

## REVIEW / SYNTHÈSE

## Fibroblast growth factors and their receptors

Zoya Galzie, Anne R. Kinsella, and John A. Smith

**Abstract:** Fibroblast growth factors (FGFs) represent a group of polypeptide mitogens eliciting a wide variety of responses depending upon the target cell type. The knowledge of the cell surface receptors mediating the effects of FGFs has recently expanded remarkably. The complexity of the FGF family and the FGF-induced responses is reflected in the diversity and redundancy of the FGF receptors. In this review, a number of biochemical characteristics and biological properties of the FGF family and its receptors are described and their expression both in normal tissues and in tumours is discussed. Finally we speculate on the targeting of growth inhibition agents to tumours through FGF receptors.

**Key words:** fibroblast growth factor, FGF receptor, heparan sulphate proteoglycans, tyrosine kinase receptors, FGF in tumour diagnosis.

**Résumé :** Les facteurs de croissance des fibroblastes (FGF) constituent un groupe de polypeptides mitogènes entraînant une grande variété de réponses en fonction du type des cellules cibles. Récemment, notre connaissance des récepteurs membranaires intervenant dans les effets des FGF s'est accrue considérablement. La complexité de la famille des FGF et des réponses induites par les FGF est reflétée dans la diversité et la redondance des récepteurs de FGF. Dans cette revue, nous décrivons certaines caractéristiques biochimiques et propriétés biologiques de la famille des FGF et de leurs récepteurs et nous discutons de l'expression des FGF dans les tissus normaux et les tumeurs. Finalement, nous spéculons sur le ciblage d'inhibiteurs de croissance sur les tumeurs par l'intermédiaire des récepteurs de FGF.

**Mots clés :** facteur de croissance des fibroblastes, FGF, récepteur de FGF, protéoglycane à sulfate d'héparane, récepteurs à activité de tyrosine-kinase, diagnostic de tumeurs.  
[Traduit par la rédaction]

### FGF family of growth factors

Fibroblast growth factors (FGFs) are members of a family of polypeptides that are potent regulators of cell proliferation, differentiation, and function (Burgess and Maciag 1989; Rifkin and Moscatelli 1989). These proteins play crucial roles in normal development (Yamaguchi and Rossant 1995; Kimmelman et al. 1988; Slack et al. 1988), in the maintenance of tissues, and in wound healing and repair (Clarke et al. 1993; Cuevas et al. 1988), and they have also been implicated in a wide range of pathological conditions, including tumorigenesis and metastasis (Davies et al. 1996; Myoken et al. 1996). They act on cells of meso-, ecto-, and endo-dermal origin, and they cause changes in migration, morphology, and function as well as proliferation (Gospodarowicz et al. 1978, 1986). While the initial discovery of these factors was as fibroblast mitogenic factors (Gospodarowicz 1974; Rudland et al. 1974), hence the name, their roles are far more extensive than that,

and not all members are, in fact, mitogenic for fibroblasts. The FGF family currently consists of nine (and probably more) members (Fig. 1 and Table 1) with 30–50% sequence homology at the amino acid level and with conservation of the positions of two cysteine residues. The factors are designated FGF-1 through FGF-9, although the names acidic FGF and basic FGF are used for FGF-1 and FGF-2, respectively, and keratinocyte growth factor (KGF) is commonly used for FGF-7. Four members of the FGF family are oncogene products: FGF-3 (*int-2*), FGF-4 (*hst-1*, KGF), FGF-5, and FGF-6 (*hst-2*). FGF-8 is also known as androgen-induced growth factor whereas FGF-9 is called the glial activating factor.

At least four distinct receptors for FGFs have been discovered (FGFR1, FGFR2, FGFR3, and FGFR4), with many additional structural variants resulting from alternative splicing (Jaye et al. 1992; Pasquale and Singer 1989). The basic structure is an extracellular region with three immunoglobulinlike domains, a transmembrane region, and a cytosolic tyrosine

Received August 12, 1997. Revised November 4, 1997. Accepted November 10, 1997.

**Abbreviations:** CS, chondroitin sulphate; FGF, fibroblast growth factor; GAF, glial activating factor; GlcA, glucuronate; HS, heparan sulphate; HSPG, heparan sulphate proteoglycan; IdoA, iduronate; KGF, keratinocyte growth factor; SH2, src homology domains.

Z. Galzie. Departments of Surgery and Biochemistry, University of Liverpool, Liverpool, L69 3BX, U.K.

A.R. Kinsella. Department of Surgery, University of Liverpool, Liverpool, L69 3BX, U.K.

J.A. Smith.<sup>1</sup> Department of Biochemistry, University of Liverpool, Liverpool, L69 3BX, U.K.

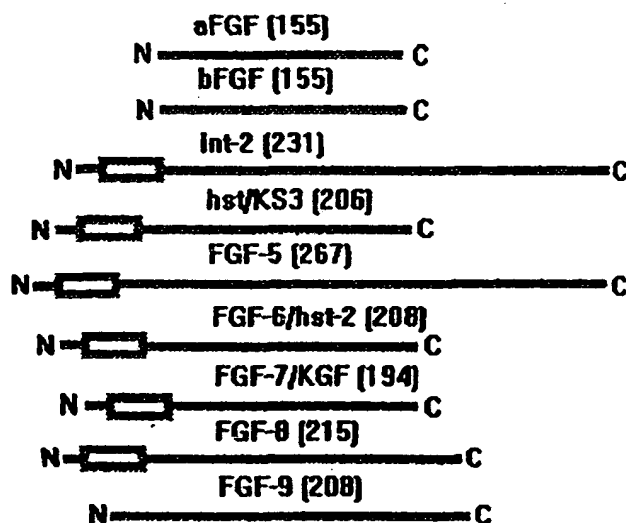
<sup>1</sup> Author to whom all correspondence should be addressed.

**Table 1.** Properties of the members of the fibroblast growth factor family.

FGF	Gene or protein pseudonyms	Chromosomal map location	Amino acid length	Signal sequence	Associated functions
FGF1	Acidic fibroblast growth factor; endothelial cell growth factor	5q31	155	No	Endothelial cell migration and proliferation; angiogenesis; delayed-early gene activation
FGF2	Basic fibroblast growth factor	4q25	155 (but has longer forms)	No	Angiogenesis; astrocytomas
FGF3	Oncogene <i>int2</i>	11q13	239 <sup>a</sup>	Yes	Mouse mammary carcinoma; formation of the inner ear spatial patterning processes
FGF4	Oncogene <i>hst</i> ; transforming factor from human stomach cancer; K-FGF	11q13	176	Yes	Human stomach cancer; melanoma; Kaposi's sarcomas; limb development
FGF5	Oncogene fibroblast growth factor 5	4q21	267 <sup>a</sup>	Yes	Inhibits hair elongation
FGF6	Oncogene <i>hst2</i>	12p13	198 <sup>a</sup>	Yes	—
FGF7	Keratinocyte growth factor (KGF)		162	Yes	Epidermal growth and wound healing
FGF8	Androgen-induced growth factor (AIGF)		193, 246	Yes	Androgen-dependent tumour cell proliferation
FGF9	Glial-activating factor (GAF)		208	Internal?	Glial cell proliferation

<sup>a</sup> Length of immature protein deduced from cDNA.

**Fig. 1.** Structural features of the FGF family. Structure of the nine FGFs that contain the regions of the molecule required for the binding to cell surface receptors. The secretory signal sequence is represented by an open box. The lengths of the primary translation products initiated from the AUG codons are also shown.



kinase domain that is activated on ligand binding. The variants have missing immunoglobulin domains or are secreted forms made up of a portion of the extracellular component of the receptor. Some of the receptors bind, and are activated by, multiple FGFs, while others have a higher degree of specificity, suggesting that a cell may determine its susceptibility to FGFs by the receptor or receptor isoforms it expresses.

The FGF–receptor interaction is complicated by the role of polyanions. FGFs have a high affinity for heparin and similar molecules and the binding causes a conformational change (Prestrelski et al. 1992) that protects FGF from proteolysis and denaturation (Gospodarowicz and Chen 1986). Only heparin-bound basic FGF will bind to soluble receptors (Ornitz et al. 1992), and the heparinlike molecules are required for binding of basic FGF to its high-affinity receptor (Yayon et al. 1991). Extracellular FGF is mostly bound to heparan sulphate proteo-

glycans of the matrix or cell surface. This binding, protecting the FGF, is essential for the proper presentation of FGF to its receptors and may represent a reservoir of FGF. Hydr lysis of the extracellular matrix can release active FGF heparan sulphate complexes (Bashkin et al. 1989), suggesting a mechanism for cellular control by heparanase, proteoglycosidase, or other enzymes.

### Prototypic FGFs

Acidic and basic FGF (aFGF and bFGF), the prototypic members of this family, were named for their differing isoelectric points. They have similar molecular weights and spectra of biological activities, and they show approximately 55% amino acid similarity. bFGF has been isolated from a variety of sources, including neural tissue, pituitary, adrenal cortex, corpus luteum, and placenta (Esch et al. 1985; Gospodarowicz et al. 1974, 1978). When isolated from natural sources, bFGF usually has an apparent molecular mass of about 18 kDa, but a variety of larger forms, up to 24 kDa, also exist as a result of amino-terminal extensions of the protein produced by initiation of translation at non-AUG start sites (Florkiewicz and Sommer 1989; Moscatelli et al. 1987; Prats et al. 1989). The larger forms are localized in the cell nucleus rather than cytoplasm (Powell and Klagsbrun 1991; Tessler and Neufeld 1990). aFGF has been found in brain, retina, bone matrix, and osteosarcomas (Gospodarowicz et al. 1986; Thomas et al. 1980). Several forms of aFGF, varying in size from 16 to 18 kDa, are generated by proteolysis during purification (McKeehan and Crab 1987). Unlike other members of the FGF family, neither aFGF nor bFGF possess an obvious signal sequence, although the receptors for FGF on cell surfaces suggest an export mechanism.

Acidic and basic FGF stimulate the proliferation of all cells of mesodermal origin, and many cells of neuroectodermal, ectodermal, and endodermal origin. The cells include fibroblasts, endothelial cells, astrocytes, oligodendrocytes, neuroblasts, keratinocytes, bovine epithelial lens cells, osteoblasts, smooth muscle cells, and melanocytes (Burgess and Maciag 1989; Klagsbrun 1989; Rifkin and Moscatelli 1989). Acidic and basic FGF are chemotactic and mitogenic for endothelial cells in vitro, inducing production of factors involved in the

breakdown of the basement membrane and the migration of capillary endothelial cells into collagen matrices to form capillarylike tubes (Gospodarowicz et al. 1987). aFGF has been implicated as a mesoderm-inducing factor in *Xenopus laevis* (Kimmelman et al. 1988) and bFGF has been shown to be crucial in modulating embryonic development and differentiation. These and other in vitro observations suggest that FGFs play a role in vivo in the modulation of such normal processes as angiogenesis, wound healing and tissue repair, embryonic development and differentiation, and neuronal function and neural degeneration (Klagsbrun 1989; Burgess and Maciag 1989). These observations, as well as the observations that inappropriate expression of bFGF and other members of the family can result in tumour production (Fernig et al. 1993; Luqmani et al. 1992), also suggest that FGFs may participate in the production of a variety of pathological conditions resulting from excessive cell proliferation and excessive angiogenesis.

### Other members of FGF family

Since the determination of the partial amino acid sequences of the aFGF and bFGF polypeptides and the characterization of the sequences of their corresponding cDNAs, seven other members of the FGF family referred to above have been discovered. Many of the other FGFs have been identified by cloning of their cDNAs in transformation experiments and it is only subsequently that their functions in normal tissues have begun to be appreciated.

#### *int-2* (FGF-3)

The product of the *int-2* gene was the third member of the FGF family to be identified (Acland et al. 1990; Smith et al. 1988; Dickson and Peters 1987). *int-2* is a cellular gene that was shown to become transcriptionally active after integration (*int*) of the mouse mammary tumour virus into the mouse genome (Dickson et al. 1984). It codes for a protein of 240 amino acids with a 44 and 38% homology to the internal core of the primary sequence of bFGF and aFGF, respectively (Fig. 1). The principal differences are the addition of a novel 16 amino acid sequence at the N-terminus and a 45 amino acid extension in the C-terminal region. The amino terminal extension contains a short noncharged sequence that acts as a signal sequence. As with bFGF, N-terminally extended forms of *int-2* are also found (Fig. 1). These extensions are translated from alternative CUG initiation codons and provide a nuclear localization sequence that directs the extended forms of *int-2* to the nucleus, overriding the secretory signal encoded from the AUG codon (Acland et al. 1990).

#### *hst/KS53* (K-FGF or FGF-4)

The fourth member of the FGF family was identified as genomic DNA fragments derived from a human stomach tumour (*hst*) and a Kaposi's sarcoma lesion capable of inducing murine 3T3 cell transformation in vitro (Delli-Bovi et al. 1988; Taira et al. 1987; Sakamoto et al. 1986). The open reading frame encodes a polypeptide 206 amino acids in length with 43, 38, and 40% amino acid sequence homology to bFGF, aFGF, and *int-2*, respectively. Again the homologous sequences correspond to the central 18-kDa portion of the bFGF polypeptide (Fig. 1). The N-terminal domain of *hst* is extended, compared with that of bFGF, by the addition of

55 amino acids that include a secretory signal peptide sequence. The *hst* polypeptide also possesses a short COOH-terminal extension. The mature protein contains 175 amino acids (23 kDa), is glycosylated, and is secreted into the medium of cells transfected with the relevant cDNA. The expression of *hst/K-FGF* is seen to increase in the mid stage of embryonic development in the mouse but is absent from normal adult tissues. *hst/KS53*, like *int-2*, is located on chromosome 11 (Yoshida et al. 1987).

#### FGF-5

FGF-5 was isolated from the DNA of a human bladder (Zhan et al. 1988). It contains 267 amino acids with an extensive hydrophobic sequence in the amino terminal region. The homology at the amino acid level to the core of aFGF and bFGF is 42 and 45%, respectively. The expression of FGF-5 is limited to neonatal brain and some human tumour lines (Klagsbrun 1989).

#### FGF-6

This oncogene was isolated from a mouse cDNA library by screening with the *hst* gene (Marics et al. 1989) and accordingly shares a 70% homology at the amino acid level to the *hst* product. FGF-6 maps to chromosome 12.

#### KGF (FGF-7)

The cDNA for KGF was isolated from keratinocytes and contains an open reading frame encoding a 194 amino acid polypeptide with an estimated molecular mass of 22 kDa (Finch et al. 1989). KGF shares 37 and 39% amino acid homology to aFGF and bFGF. The N-terminus of KGF is extended compared with the core of bFGF and contains a putative hydrophobic secretion signal. KGF mRNA is present in a number of stromal fibroblast cell lines derived from embryonic neonatal and adult sources but is absent from a variety of normal glial cells and epithelial cell lines (Finch et al. 1989). It was originally thought that the activity of KGF was specific for keratinocytes and epithelial cells (Rubin et al. 1989). However, it has since become apparent that in adult tissues the underlying theme of KGF expression is that it is produced by stromal fibroblasts but acts on the cells of the epithelium (Colemankrnacik and Rosen 1994; Ron et al. 1993; Taylor et al. 1993).

#### FGF-8

Androgens were found to induce the secretion of a number of bFGF-like growth factors by an androgen-dependent mouse mammary carcinoma cell line, Shionogi carcinoma-3 (Tanaka et al. 1992). These bFGF-like growth factors exhibited a significant autocrine growth stimulating property on the Shionogi carcinoma-3 cells (Nonomura et al. 1990). One of these activities was isolated and characterized as FGF-8, which shares 30–40% homology at the amino acid level with the other members of the FGF family (Tanaka et al. 1992) (Fig. 1).

#### FGF-9

FGF-9 is sometimes referred to as glial-activating factor (GAF) and was originally purified from the culture supernatant of a human glioma cell line (Miyamoto et al. 1993). Its amino acid homology to the other members of the FGF family is around 30%. Like aFGF and bFGF, it has no secretory signal

sequence, although it is released from cells (Miyamoto et al. 1993).

### Structure-function relationship in FGFs

Using a series of synthetic peptides, two regions of a 155 amino acid bFGF have been proposed to be involved in mediating receptor binding and hence mitogenic activity on different cell types, including fibroblasts, neural cells, and endothelial cells. These regions cover amino acid residues 33–77 and 115–124 of bovine bFGF and the corresponding synthetic peptides act either as partial bFGF agonists or as antagonists in DNA synthesis assays on 3T3 fibroblasts (Baird et al. 1988). Moreover, the binding of bFGF to cellular receptors is inhibited by a peptide corresponding to the amino acid sequence 112–155 of bFGF, but not by the region of amino acid residues 33–77, suggesting that the region of amino acid residues 115–124 represents the core sequence required for the binding of bFGF to its cellular receptors (Baird et al. 1988; Walicke et al. 1989). In another study two synthetic peptides encompassing amino acid residues 38–61 and 82–101 have been investigated (Presta et al. 1991). Both peptides have a weak agonist effect and are able to antagonize the stimulation of cell growth by bFGF. Antibodies raised to these synthetic peptides are able to specifically inhibit the growth-stimulatory effects of bFGF. However, the fact that high concentrations of synthetic peptides (100–300 µg/mL) were used in the above studies does raise the possibility that some of the results may be experimental artefacts.

The analysis of the activity of various recombinant mutant bFGFs suggests that the above results obtained with synthetic peptides may not be entirely correct. Other important sequences located at the amino terminal region of bFGF (residues 27–32) (Isacchi et al. 1991) have been found to mediate the biological activity of bFGF. In addition a recombinant mutant has been produced in which basic residues Arg-118, Lys-119, Lys-128, and Arg-129 have been replaced with neutral glutamine residues. This mutant bFGF (M6B-bFGF) has been used for the detailed assessment of the role exerted by these four basic amino acids in mediating the biological properties of bFGF (Presta et al. 1992). When compared with wild-type bFGF, the M6B-bFGF mutant lacked significant angiogenic activity *in vivo*. However, in cultured endothelial cells, it demonstrated a similar receptor-binding capacity and mitogenic activity, but a reduced affinity for low-affinity heparan sulphate (HS) binding sites, a reduced chemotactic activity, and a reduced capacity to induce the production of urokinase-type plasminogen activator (Presta et al. 1992). Seno et al. (1990) produced an exhaustive set of C-terminal deletion mutants of bFGF and concluded that the residues between positions 116 and 141 played a major role in the mediation of the binding of bFGF to heparin. However, the stability and structure of the bFGF mutants has not been examined. Thus, it is not clear whether the loss of activity of the mutant bFGFs is due to the modification of their amino acid sequence or due to the destabilization of their structure.

A fragment of aFGF (N terminal-122) cleaved by thrombin has a significantly lower affinity for immobilized heparin than wild-type aFGF and is at least 50 times less potent at stimulating mitogenesis (Lobb 1988). Moreover, reductive methylation of aFGF reduces its capacity to stimulate mitogenesis in

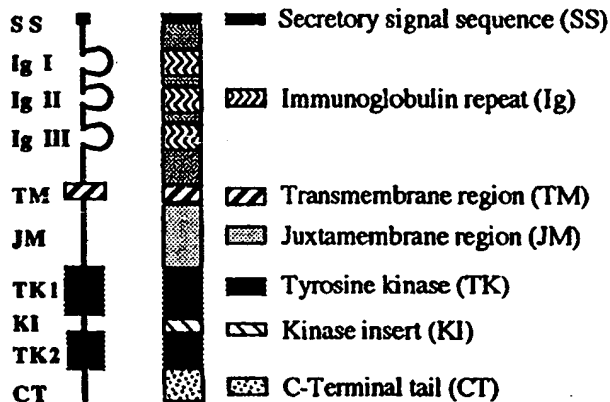
Balb/c 3T3 cells, and this correlates with the modification of less than 3 of its 12 lysine residues, one of which is Lys-119, at the C-terminal of aFGF (Harper and Lobb 1988). The methylated aFGF has a decreased affinity for heparin and is a considerably less potent growth factor. The results indicate that the C-terminal region of aFGF is functionally important in both mitogenesis and heparin binding (Lobb 1988). Moreover, these results also show that in aFGF, Lys-119 plays an important role in heparin binding and suggest that this residue and its local environment are involved both in the interaction of aFGF with heparin and in the delivery of a growth-stimulatory response (Harper and Lobb 1988). Since the sequences of aFGF and bFGF are highly conserved in this region, it may be that the corresponding Lys in bFGF is similarly involved in binding to cellular receptors.

The crystal structures of aFGF and bFGF have been resolved by X-ray crystallography. The overall structure is a cylindrical barrel made up of 12 anti-parallel  $\beta$ -strands. bFGF exhibits an approximate 3-fold internal symmetry about the axis of the  $\beta$ -barrel (Ago et al. 1991; Eriksson et al. 1991; Zhang et al. 1991). Ago and coworkers (1991) calculated the electrostatic potential on the solvent-accessible surface of bFGF to investigate the heparin-binding sites and they found a highly positively charged region consisting of a  $\beta$ -turn (Lys-119 to Trp-123), a  $\beta$ -strand (Tyr-124 to Arg-129), a short  $\beta$ -strand (Tyr-133 to Lys-138), and a loop (Thr-139 to Leu-147). This region includes five basic residues (Lys-128, Arg-129, Lys-134, Lys-138, and Lys-144) but no acidic residues and correlates with the region identified with synthetic peptides, protein modification, and recombinant mutant proteins.

### Receptors for bFGF

[<sup>125</sup>I]FGF binding experiments on Swiss 3T3 cells and mouse skeletal muscle myoblasts revealed the existence of two distinct binding sites with dissociation constants of 1–100 pM and 1–10 nM, respectively (Olwin and Hauschka 1986; Neufeld and Gospodarowicz 1985). The use of serial salt washes or competition with heparin suggested that the low-affinity receptors were heparan sulphate proteoglycans (HSPGs) (Moscatelli 1987; Olwin and Hauschka 1986). However, the use of Scatchard analysis, in these and other studies, to quantify FGF binding data is invalid because the binding data are nonsaturating (Klotz 1982). Moreover, the use of serial salt and acid washes to distinguish quantitatively the high- and low-affinity receptors has been found to be erroneous because both binding sites are, in part, salt and acid sensitive (Fernig et al. 1992; Kan et al. 1988). Nevertheless, the low-affinity binding of bFGF is likely to be due to HSPGs because it is sensitive to heparinase and competition by exogenous heparin in all cells studied so far (Fernig et al. 1992; Kan et al. 1988). Indeed, a bFGF-binding HSPG, syndecan-1, has been isolated by expression cloning as a low-affinity receptor for bFGF (Kiefer et al. 1990). The high-affinity binding site was originally identified as a tyrosine kinase. cDNAs encoding these molecules, called FGFR, have subsequently been isolated and sequenced. However, it is not now certain whether, on their own, the tyrosine kinase receptors represent the high-affinity binding site.

**Fig. 2.** General structure of FGFRs. Schematic structure of the FGFRs, the fourth family of tyrosine kinase receptors. The FGFRs are encoded by four distinct genes: FGFR-1 (*flg*), FGFR-2 (*bek*), FGFR-3, and FGFR-4.



### Tyrosine kinase receptors (FGFRs)

The FGFR are one of nine subclasses of tyrosine kinase receptors (Fantl et al. 1993; Ullrich and Schlessinger 1990). The FGFRs (Jaye et al. 1992) are divided into four distinct types, each encoded by a separate gene: FGFR-1 (*flg* or *fms*-like gene; Pasquale and Singer 1989), FGFR-2 (*bek* or bacterial expressed kinase; Houssaint et al. 1990), FGFR-3 (Keegan et al. 1991), and FGFR-4 (Partanen et al. 1991).

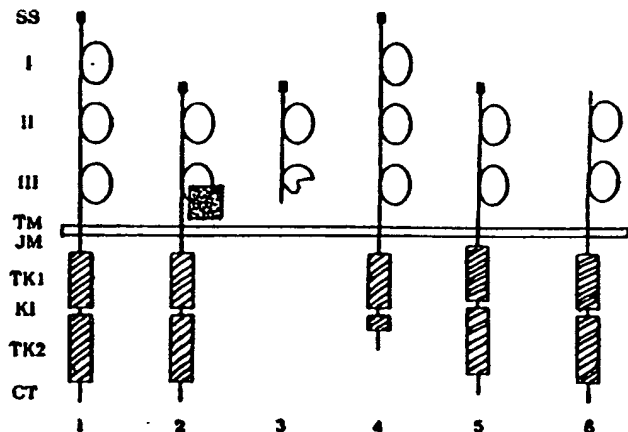
#### General structure of FGFRs

The FGFRs contain a single membrane-spanning domain, an extracellular region, and an intracellular region. The extracellular region of FGFRs is characterized by three consensus immunoglobulinlike loops. Between immunoglobulinlike loops 1 and 2 is a short domain referred to as the acidic box domain, which is unique to the FGFRs (Fig. 2). In the FGFR-1 protein, the acidic box domain contains a core sequence of eight consecutive acidic residues. The intracellular region of the FGFRs possesses a relatively long juxtamembrane domain and a tyrosine kinase domain that is split by a kinase insert (Fig. 2). Following the tyrosine kinase domain is the C-terminal tail domain, which is relatively divergent in sequence between the four FGFRs and approximately 55–66 amino acids long. Whilst not all FGF–FGFR binding interactions have been studied, it would appear that most FGFs will bind to any FGFR. For example, FGR-1 binds aFGF, bFGF, *int*-2, *hst*, and FGF-8, but not FGF-7, while FGFR-2 binds aFGF, bFGF, *hst*, and FGF-7, but not FGF-5 (Fernig and Gallagher 1994; Mansukhani et al. 1992).

The complexity of the FGFRs system is further increased by differential RNA splicing, which is dictated by the exon structure of the genes encoding the FGFRs (Fig. 3). This exon structure, which is conserved in FGFR-1, -2, and probably -3, includes the three alternative variants of exon 6, which encodes the third immunoglobulin loop (Dell and Williams 1992; Duan et al. 1992). In FGFR-4, the structure is similar except that there are no alternatively spliced variants (Vainikka et al. 1992).

Six of the different FGFR-1 proteins, FGFR-1-I through -VI, produced in this manner are shown in Fig. 3. The differences in the isoforms of FGFR-1 are as follows (Fig. 3): (i) no

**Fig. 3.** Exon splicing generates multiple isoforms of FGFRs. Generation of FGFR-1 receptor subtypes by alternative splicing. Six of the 48 different isoforms of FGFR-1 are shown. The region of Ig-loop III encoded by exon 6 is enclosed by a shaded box. Splice forms II and VI differ by just two amino acids in their extracellular domains.



secretory signal sequence (FGFR-1-VI); (ii) deletion of the first immunoglobulin loop (FGFR-1-II, -III, -V, and -VI); (iii) three alternative C-terminal parts of immunoglobulin-loop III (shaded box, FGFR-1-II), encoded by three different exon 6 including one with a stop codon that results in the synthesis of a secreted receptor (FGFR-1-III); (iv) deletion of just two amino acids between the third immunoglobulin loop and the transmembrane domain (FGFR-1-II and -V); and (v) a truncated tyrosine kinase domain (FGFR-1-IV). Similar splicing events generate isoforms of FGFR-2 (Champion-Arnaud et al. 1991) and FGFR-3 (Murgue et al. 1994). Thus these are a total of 48 isoforms of FGFR-1, FGFR-2, and FGFR-3, making a total of 145 different FGFR proteins.

#### Functions of FGFR isoforms

The isoforms of FGFR-1 and FGFR-2 that vary in the third immunoglobulin loop region have different ligand-binding specificities. The secreted form of FGFR-1, FGFR-1-III, which uses a different exon for immunoglobulin loop 3 to the membrane-bound forms (Fig. 3), binds bFGF but not aFGF with high affinity (Duan et al. 1992). The membrane-bound forms of FGFR-1 (Fig. 3) may either bind aFGF and bFGF with equal affinity or only bind aFGF with high affinity depending on which of the remaining two exons encoding the third immunoglobulin is used (Werner et al. 1992) (Fig. 3). The three exons that encode the third immunoglobulin loop of FGFR-2 also confer a degree of ligand specificity. One of the three splice variants binds FGF-7 and aFGF but not bFGF with high affinity, whilst a second splice variant binds aFGF and bFGF but not FGF-7 with high affinity (Champion-Arnaud et al. 1991). Detailed analyses of the contributions of the second and third immunoglobulin loops indicate that in this case both contribute to specifying the high-affinity ligand for FGFR-2 (Cheon et al. 1994; Zimmer et al. 1993).

The isoforms produced from the exon 6 variant possessing a stop codon may act as classic binding proteins or as soluble receptor antagonists (Duan et al. 1992). Similarly, the isoforms with a truncated tyrosine kinase (Fig. 3) may act as

dominant negative antagonists. There is also evidence that some isoforms of the FGFRs serve to alter the binding specificity of the receptors, and that different FGFRs may have different signalling roles. For example, the immunoglobulin loop isoforms of FGFR-1 appear to be associated with the transformed and the malignant phenotypes (Yamaguchi et al. 1994; Yan et al. 1992). The secreted and kinase-truncated isoforms of FGFR-1 may act as antagonists (Duan et al. 1992).

Thus alternative RNA splicing imparts some degree of specificity to the FGF-FGFR binding reaction but there is still considerable overlap between the binding of the nine FGFs and the different FGFRs and their isoforms. However, the reason for the existence of the estimated 145 different FGFRs is unclear at present.

### Signalling by FGFR tyrosine kinase

The FGFRs, like the other tyrosine kinases, have an extracellular ligand-binding domain and an intracellular domain, which includes the protein tyrosine kinase, separated by the plasma membrane. The activation of the receptors, caused by the binding of an extracellular ligand, must be translated across the membrane barrier into the activation of the signal transduction functions of the intracellular domain. It is thought that the binding of FGFs to the extracellular domain induces the dimerization of the FGFRs, which stabilizes interactions between adjacent cytoplasmic domains and leads to activation of the tyrosine kinase function (Jaye et al. 1992; Ullrich and Schlessinger 1990). The dimerization of FGFRs is followed by receptor autophosphorylation, which mainly occurs by one receptor molecule phosphorylating the other in the dimer (Jaye et al. 1992). The phosphorylation of the tyrosine kinase receptor increases the activity of the enzyme and activates the binding sites for proteins that are targets of the receptor tyrosine kinase. This mechanism of ligand-induced receptor dimerization is thought to be common to the entire family of receptor tyrosine kinases (Fantl et al. 1993; Ullrich and Schlessinger 1990).

After ligand binding, receptor dimerization, and the activation of tyrosine kinase, the receptors cluster into clathrin-coated pits and are internalized via receptor-mediated endocytosis (Fantl et al. 1993). Individual phosphotyrosine residues present in the cytoplasmic domains of FGFRs serve as highly selective binding sites that interact with specific cytoplasmic molecules. These signalling molecules may mediate the cellular responses to growth factors. The association between the tyrosine phosphorylated regions in growth factor receptors and signalling proteins is mediated in some cases by a conserved region of approximately 100 amino acids, termed *src* homology domains (SH2) (Anderson et al. 1990). Tyrosine phosphorylation of the binding site serves as the receptor for SH2 association, while the C-terminal residues provide specific recognition of the relevant SH2 domain containing target protein (Songyang et al. 1994). SH2 domains are usually accompanied by another conserved domain of 50 amino acid residues termed the SH3 domain (Musacchio et al. 1992), which may extend the ability of signalling proteins to complex with one another (Fig. 4).

### Heparan sulphate proteoglycan

The HSPGs have been shown to be the low-affinity receptors

for bFGF (dissociation constant of 10–100 nM). The HSPGs are the most complex and variable of the mammalian proteoglycans and consist of a core protein to which a variable number of glycosaminoglycan chains are linked (Gallagher et al. 1990) (Fig. 5). The carbohydrate components of proteoglycans are glycosaminoglycans that consist of repeating disaccharide units that can be substituted with ester and amino sulphate groups. These substitutions make the glycosaminoglycans highly anionic molecules. In HSPGs the glycosaminoglycan is HS. The HSPGs are widely expressed on diverse cell and tissue types including liver cells. In addition, the extracellular matrix, which also contains HSPG, can bind and act as a reservoir for the FGFs (Vlodavsky et al. 1991; Baird and Ling 1987). These facts pose interesting targeting, pharmacokinetic, and biodistribution challenges for molecules targeted by the FGFs and they constitute one reason the serum half-life of bFGF and FGF-targeted toxins is so short (Beitz et al. 1992; Whalen et al. 1989). HSPGs readily internalize (Yanagishita and Hascall 1984) and are a method of cell entry of growth factor – toxin conjugates. However, it has been reported that internalization of FGF-SAP by this route does not result in cytotoxicity, presumably because it does not have access to ribosomes for protein synthesis inhibition (Reiland and Rapraeger 1993). Interference with the FGF-HSPG interaction has been used to inhibit tumour growth.

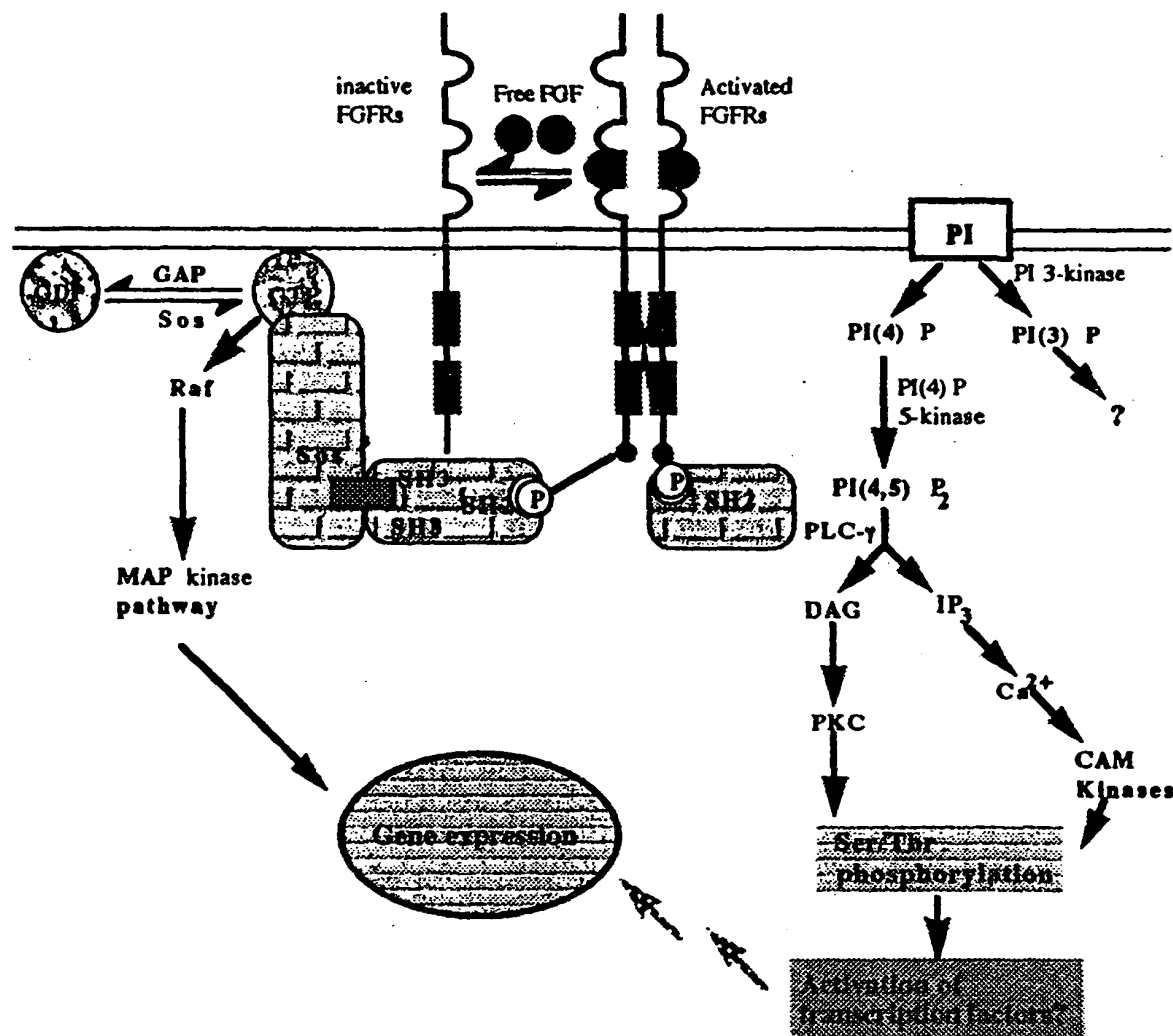
### HSPG core proteins

#### Syndecans

Syndecan-1, originally isolated from mouse mammary epithelial cells, was the first core protein to be characterized (Saunders and Bernfield 1988; Koda et al. 1985). Syndecan-1 is a composite proteoglycan containing both HS and chondroitin sulphate (CS) glycosaminoglycan chains (Rapraeger et al. 1985). A cDNA encoding syndecan-1 was subsequently isolated and predicted a polypeptide of 311 amino acids (32 kDa) containing a 25 amino acid transmembrane domain, a 34-residue cytoplasmic domain, and an extracellular protease-sensitive site near the transmembrane domain (Saunders et al. 1989). Two pairs of Ser-Gly dipeptide sequences to which HS or CS chains may be attached are located near the amino terminus and the transmembrane domain. The subsequent isolation of a human cDNA encoding syndecan-1 showed that the cytoplasmic and the transmembrane domains, as well as the extracellular glycosylation sites, were highly conserved. There was only one amino acid difference between the 59 residues in these regions of human and mouse syndecan-1 (Mali et al. 1990).

On the basis of the high homology of their predicted amino acid sequences, there are currently three other members of the syndecan family of HSPGs. cDNAs encoding fibroglycan (syndecan-2) have been isolated from human fetal lung fibroblasts (Marynen et al. 1989) and rat liver (Pierce et al. 1992). A cDNA encoding N-syndecan (neural syndecan or syndecan-3) has been isolated from chick embryo limb buds (Gould et al. 1992) and newborn rat Schwann cells (Carey et al. 1992). A cDNA encoding ryudocan (syndecan-4) is expressed in 14-day chick embryos (Baciu et al. 1994) and has also been reported in rat microvascular endothelial cells, smooth muscle cells, and skin fibroblasts (Kojima et al. 1992). The extracellular domain is responsible for virtually all of the

**Fig. 4.** Signalling events initiated by tyrosine kinase of FGFRs. Binding of FGF to FGFRs is followed by rapid dimerization, tyrosine kinase activation, and phosphorylation of signalling molecules such as phospholipase C- $\gamma$  and GTPase activating protein (GAP). PLC- $\gamma$  and GTPase activating protein bind to phosphorylated regions in growth factor receptors through their src-homology domain.



structural differences between the members of the syndecan family. The conservation of the transmembrane and intracellular domains suggests that they are important for the function of the proteoglycans, although how this may occur is not yet known.

#### Glypicans

In 1990, David and coworkers isolated and sequenced cDNA clones for the core protein of a HSPG from human lung fibroblasts (David et al. 1990). The core protein structure and the effects of phospholipase C treatment suggest that this proteoglycan is linked to the cell surface via a glycosylphosphatidylinositol anchor. This proteoglycan is rapidly, quantitatively, and selectively shed from the cell surface by cultured fibroblasts and they referred to this glypiated proteoglycan by the name glypican (David et al. 1990).

#### Perlecan

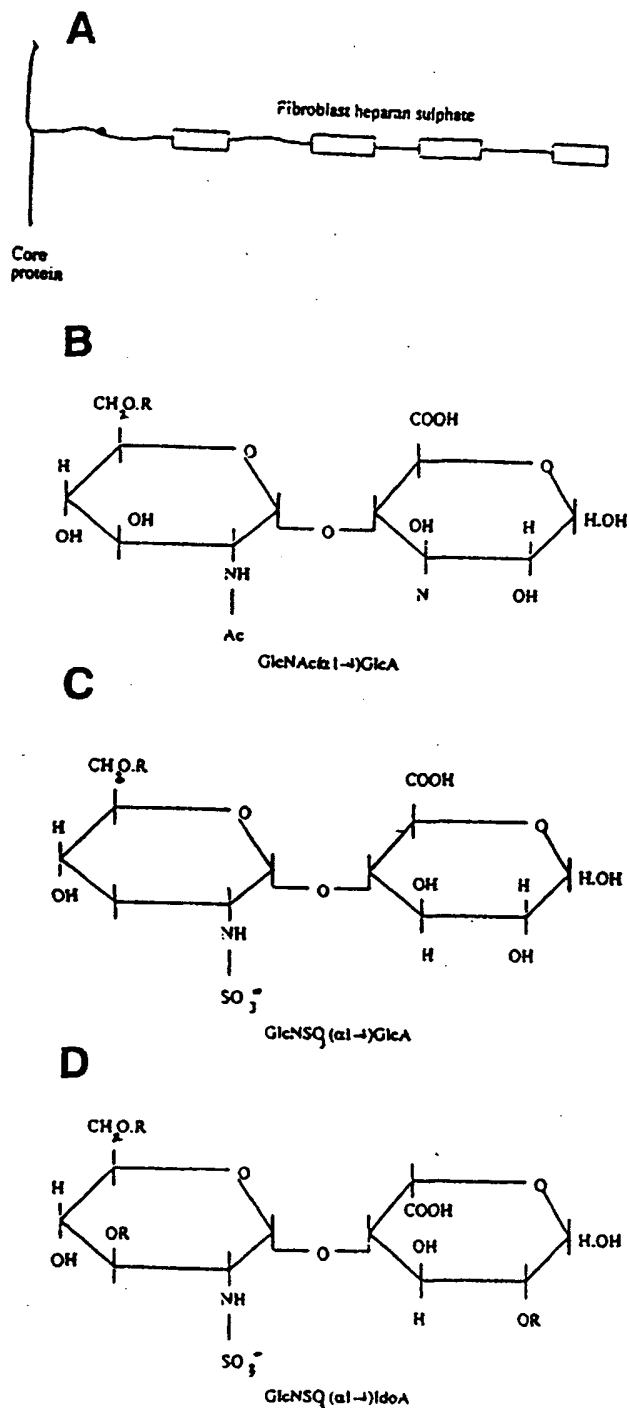
Perlecan, the main proteoglycan of basement membranes and pericellular spaces, contains one of the largest single-chain

polypeptides of vertebrates. Perlecan has a core protein of 400 kDa and analysis of cDNA clones from mouse and human indicates the presence of conserved domains that have sequence homology to laminin  $\beta$ 1 and  $\beta$ 2 chains (Noonan et al. 1988). It also contains structures analogous to a multiloop sequence found in the neural cell adhesion molecule (N-CAM) and a domain found in the low-density lipoprotein (LDL) receptor. In addition to basement membranes, perlecan is also found in the stroma surrounding tumours (Murdoch et al. 1992) and has been isolated from fibroblast cultures (Heremans et al. 1989).

#### CD44

CD44 is a polymorphic family of integral membrane glycoproteins of which the smallest form (80–90 kDa) contains no detectable glycosaminoglycan. It was originally identified on the surface of lymphocytes where it is involved in the process of lymphocyte homing and lymphohemopoiesis. A 200-kDa form contains CS chains (Jalkanen et al. 1988). CD44 is also found upon other leukocytes, epithelial cells, fibroblasts, glial

**Fig. 5.** (A) Structure of heparan sulphate proteoglycans. HSPGs consist of heparan sulphate (HS) polysaccharide chains covalently linked to core proteins. HS consists of regions of low sulphation and regions of high sulphation. Most HS-binding proteins, including FGFs, bind to the highly sulphated regions. (B–D) Disaccharide units of heparan sulphate. The hexuronate is present either as glucuronate, GlcA (B, D), or as its C-5 epimer iduronate, IdoA (D). The glucosamine may be N-acetylated (B) or N-sulphated (C, D).



cells, smooth muscle cells, and kidney mesangial cells where it is thought to play a role in cell-matrix interactions (Brown et al. 1991).

### Betaglycan

Betaglycan is primarily known as one of the three types of cell surface receptors for transforming growth factor beta. The type III receptor (betaglycan) may carry HS chains but their function is unclear as they are not necessary for transforming growth factor beta binding or storage of the growth factor at the cell surface (Cheifetz and Massague 1989). Betaglycan can also bind basic fibroblast growth factor through the HS chains, and the addition of this growth factor to cells can modify the HS content of betaglycan (Andres et al. 1992).

### Heparan sulphate

HS consists of a disaccharide repeat of  $\alpha,\beta$ -linked glucosamine and hexuronic acid (linkage sequence (D-glucosaminyl-( $\beta$ 1-4)  $\beta$ -D-hexuronosyl)<sub>n</sub>;  $n = 50-150$ ). The hexuronate is present either as glucuronate (GlcA) or as its C-5 epimer, iduronate (IdoA) (Figs. 5B–5D). HS is highly segregated to form distinct domains, regions of low sulphation that are separated by regions of high sulphation (Turnbull et al. 1992) (Fig. 5A). In regions of high sulphation the main repeat unit is N-sulphated glucosamine (GlcNSO<sub>3</sub>) and IdoA with a variable degree of substitution with ester (O) sulphates at C-6 of GlcNSO<sub>3</sub> and C-2 of IdoA; occasionally GlcNSO<sub>3</sub> is also sulphated at C-3. In regions of low or zero sulphation the glucosamine is N-acetylated (GlcNAc) and linked to GlcA, and arrays of up to 10 GlcNAc–GlcA repeats are interspersed between the sulphated domains (Turnbull and Gallagher 1988).

Heparin is composed of the same carbohydrate structures as HS but it has a significantly higher sulphate content and the main disaccharide is GlcNSO<sub>3</sub>,6S-IdoA,2S. It lacks the non-sulphated segments found in HS, is a specialized product of the mast cell, and is not a component of the cell surface (Lane et al. 1989).

### Functions of receptors for FGFs

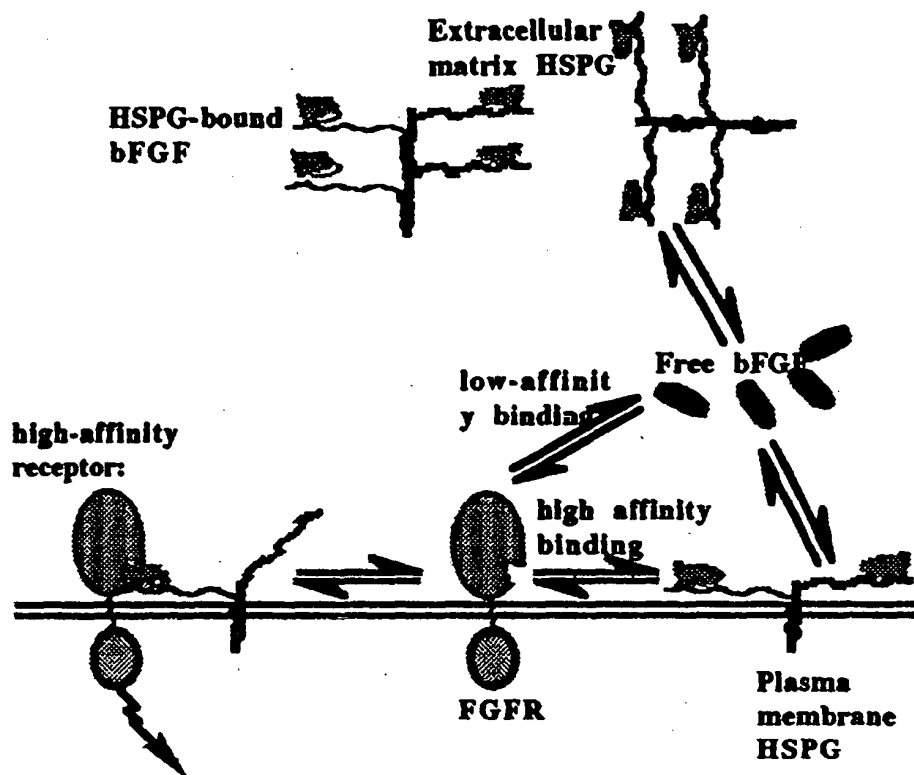
#### Storage-sink function of HS

An ever-growing number of proteins has been found to bind to HS. The HS-binding proteins include the blood coagulation factor antithrombin III, an enzyme, lipoprotein lipase, components of the extracellular matrix – basement membrane, and a large number of structurally unrelated growth factors. On the basis of the functional significance of their interactions with HS, these proteins can be divided into two groups: (i) proteins that bind HS without changing their activity and (ii) proteins that, as a consequence of binding to HS, undergo a change in activity and (or) function. Since there are large amounts of HS on the plasma membrane of cells and the surrounding extracellular matrix and basement membrane, one function of HS is to provide an extracellular site of attachment for HS-binding proteins. In the case of the extracellular matrix proteins, this function presumably relates to cell adhesion (Kallunki and Tryggvason 1992). In the case of growth factors this function may provide an extracellular storage site or sink that acts to sequester and hence inactivate growth factors.

There is considerable evidence for the HSPG receptors act-



**Fig. 6.** Dual receptor systems for FGFs. The binding of FGF to its high-affinity receptor is mediated by heparan sulphate proteoglycans (HSPG). The FGF unbound by the HSPG has a much lower or no affinity for its high-affinity receptor. Upon binding to the HSPG, the growth factor is enabled to bind the high-affinity receptor. The figure implies a conformational change, but this is not necessarily the case; there may be an optimal presentation to the receptor that is configured by the HSPG. Binding to the high-affinity receptor and receptor dimerization initiate the signal cascade.



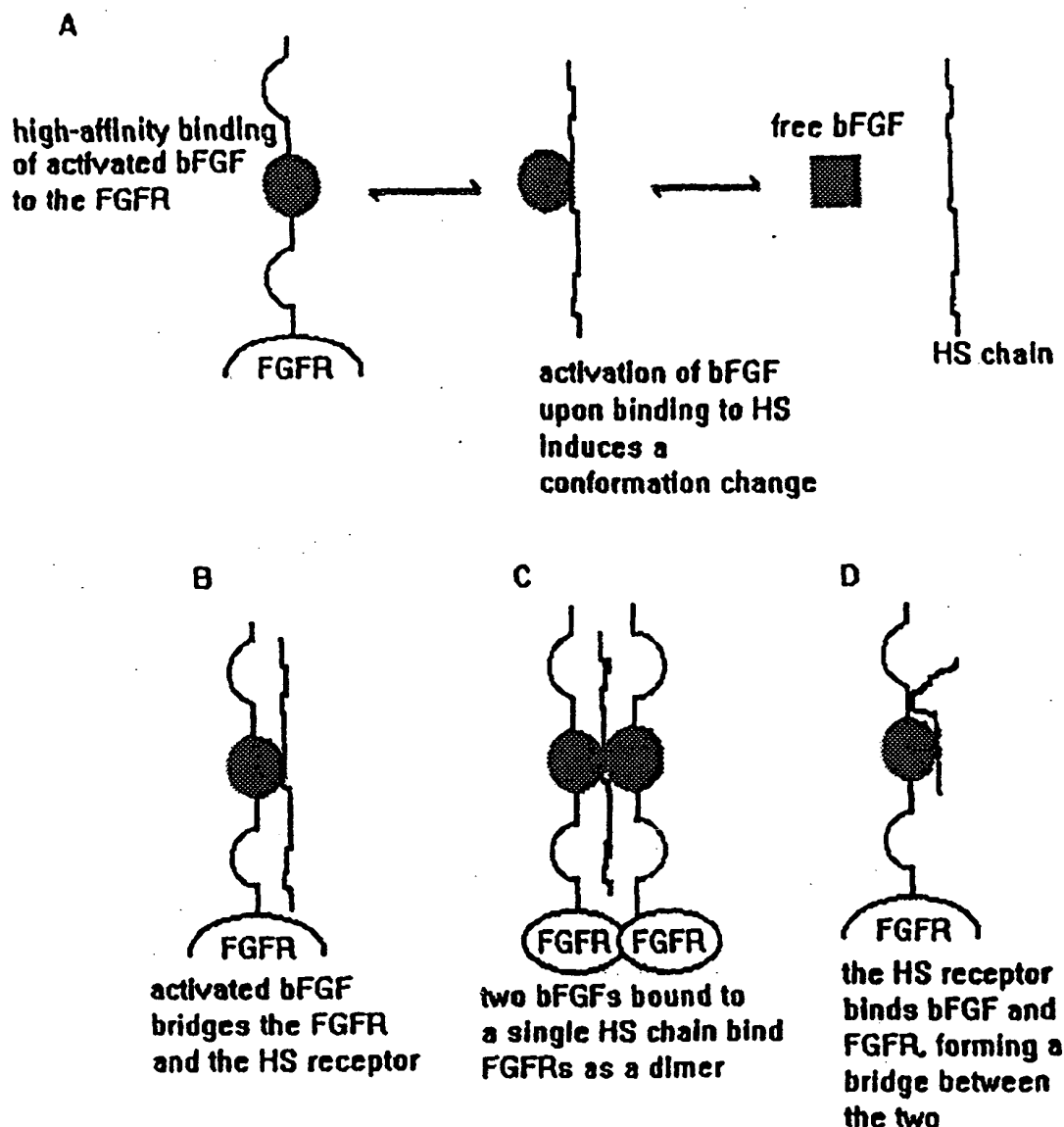
ing as storage sites for bFGF. Localization of bFGF in cultured cells and in a variety of tissues has demonstrated an association with basement membrane (Clarke et al. 1993; Barraclough et al. 1990), particularly in the vicinity of quiescent cells (Rudland et al. 1993). There are a large number of spare HS binding sites for bFGF in quiescent regions of the developing mammary gland and kidney (Morita et al. 1994; Rudland et al. 1993), suggesting that the basement membrane may act as a sink for bFGF. One of the roles of this sink may be to restrict the diffusion of bFGF and to prevent bFGF and other HS-binding growth factors from moving between cellular compartments.

Studies on cultured cells indicate a modulatory role for the storage-sink function of the HS receptor for bFGF. Cells may be loaded with bFGF for a short time, and then exogenous bFGF removed. However, the bFGF bound to cellular HS receptors remains so and is delivered slowly, but constantly, to the FGFRs, allowing a cellular response over a prolonged period of time (Fernig et al. 1992; Flaumenhaft et al. 1989). The structures within HS responsible for binding antithrombin III, bFGF, and HGF – scatter factor are different, and in the case of bFGF, there are potentially three binding sites within HS. Thus it is likely that each HS-binding protein possesses (a) unique binding structure(s) within HS. Hence the sequence of HS chains produced by cells will determine which, if any, of the HS-binding proteins are able to interact with the cells.

#### Dual receptor system

In addition to the storage and sequestration function of HS, it is also required for the activity of some HS-binding proteins. Two different experimental systems were employed to demonstrate that the growth-stimulatory activity of bFGF requires HS. Firstly, cells devoid of HS and FGFRs were transfected with a cDNA encoding FGFR-1. Cells expressing FGFR-1 could only be stimulated to grow by bFGF if exogenous heparin or HS was provided (Yayon et al. 1991). The second approach was to use cells that expressed endogenous bFGF-binding FGFR and HSPGs. The synthesis of the endogenous HS was inhibited by chlorate, and under these conditions bFGF could only stimulate cell division when exogenous heparin or HS was provided (Rapraeger et al. 1991) (Fig. 6). The original model proposed to account for these observations suggested that bFGF alone bound with only low affinity to the FGFRs. However, in the presence of heparin, bFGF bound with high affinity to the FGFRs (Rapraeger et al. 1991; Yayon et al. 1991). Subsequently, a number of other models for the dual receptor have been proposed. These fall into two classes: models in which the role of the HS is not to activate bFGF, but to bind two bFGF molecules, thereby producing a dimeric ligand that can readily induce dimerization of the FGFRs (Fig. 7A), and models in which the HS interacts with both the bFGF and the FGFR (Figs. 7B–7D). The structures within HS that may participate in the dual receptor system have been

**Fig. 7. Models of binding of bFGF to its receptors. (A)** The binding of bFGF to the HS receptor induces a conformational change in bFGF. The activated bFGF is able to bind to the FGFR with high affinity. The activated bFGF dissociates from the HS receptor prior to binding the FGFR or just after and hence the HS receptor is not part of the high-affinity receptor. **B** is similar to A except that after binding to the FGFR, the HS receptor remains bound to bFGF and hence the HS receptor is part of the high-affinity receptor. **(C)** The function of the HS receptor is to form bFGF dimers. **(D)** The HS receptors binds both bFGF and the FGFR.



examined. To date no structure has been elucidated. However, composition studies indicate that a saccharide fragment of at least 10–12 sugar residues is required and that such fragments are substantially enriched in GlcNSO<sub>3</sub> and IdoA(2S) (Walker et al. 1994; Guimond et al. 1993). Thus it would seem that these active sites in HS are similar to Oligo-H.

### Stimulation of DNA synthesis by FGFs

Addition of bFGF to cells possessing a functional dual receptor system for FGFs rapidly induces a myriad of early events. These include the stimulation of the phosphorylation on tyrosine of the FGFRs and target proteins, the formation of diacylglycerol, the activation of protein kinase C, and the mo-

bilization of Ca<sup>2+</sup> (Jaye et al. 1992). However, the role of such early events in stimulating cell division is not clear. Thus whilst it has been proposed that growth factors act as either competence or progression agents (Pledger et al. 1978), subsequent experimental evidence has not concurred with this model (Chana and Smith 1991; Rudland and Jimenez de Asua 1979). Instead it would appear that a prolonged delivery of signal(s) to cells is required to stimulate cell division. Thus, not all of the early events are initiated by any particular growth factor acting on a particular target cell and the early events in themselves are not sufficient to induce DNA synthesis, as synthesis does not take place if the growth factor is removed from the cells before the end of the lag period (Chana and Smith 1991). Furthermore, conditions are known under which some

of the early events take place, but DNA synthesis does not occur.

The interaction of most polypeptide growth factors with their cellular receptors results ultimately in the rapid degradation of the growth factors in lysosomes (Haigler et al. 1980). At least two of the FGFs, aFGF and bFGF, are notable exceptions to this rule. aFGF is recovered as a polypeptide of 42 kDa following its interaction with its cellular receptors. The 42-kDa polypeptide results from the postreceptor covalent association of aFGF with a 20-kDa protein (Shi et al. 1991). In addition, there is strong evidence to suggest that the postreceptor translocation of aFGF to the cytoplasm and thence the nucleus is a required part of the signalling mechanism that results in DNA synthesis (Wielocha et al. 1994; Imamura et al. 1992). In contrast, the interaction of bFGF with its cellular receptors produces a variety of truncated polypeptides in endothelial cells (Baldin et al. 1990; Bouche et al. 1987). Moreover, there is evidence to suggest that at least in endothelial cells, bFGF and the polypeptides that are produced as a consequence of the postreceptor processing of bFGF are translocated into the cell cytoplasm and into the nucleus (Amalric et al. 1994; Hawker and Granger 1992). Indeed, it has been suggested that the translocation of these molecules into the nucleus, and perhaps the nucleolus, is an essential growth-stimulatory signal that allows the G<sub>0</sub>-S phase transition (Bouche et al. 1987).

To elucidate how FGFs may stimulate cell division, there is clearly a need to define more precisely the conditions under which DNA synthesis takes place. It may then be possible to determine which of the many signals initiated by the interaction of FGFs with their receptors are necessary to promote cell division.

## R lease of FGF from cells

A feature of all forms of bFGF, aFGF, and FGF-9 is that they lack a consensus signal peptide for secretion whilst other members of the FGF family possess secretory signal sequences (Fig. 1). The lack of a secretory signal sequence in bFGF, aFGF, and FGF-9 and the absence of bFGF immunoreactivity in the secretory pathway suggests that these proteins are not secreted by the conventional endoplasmic reticulum – Golgi pathway (Burgess and Maciag 1989). Thus the identification of extracellular bFGF and aFGF indicated an association with the extracellular matrix in culture cells (Barracough et al. 1990; Vlodavsky et al. 1987) and tissues (Rudland et al. 1993; Gomm et al. 1991; Gonzalez et al. 1990). However, whether bFGF and aFGF were actually released from cells was controversial because of the difficulty in identifying the growth factors in the culture medium, a consequence of their binding to HS on the cell surface and in the extracellular matrix. Hence it has been suggested that bFGF and aFGF are only released from dying and dead cells and that under normal circumstances these growth factors are intracellular. However, some studies indicated that aFGF and bFGF could also be recovered from the medium of cultured cells (Jouanneau et al. 1994; Mignatti and Rifkin 1991; Bunnag et al. 1991). Consequently cell injury has been suggested to be the mechanism of aFGF and bFGF release *in vivo*. For example, the migration of endothelial cells following irradiation injury (Witte et al. 1989) *in vitro* could be inhibited by the addition of neutralizing anti-bFGF antibodies as well as protamine sulphate or suramin, which blocks the

interaction of bFGF with its receptors (Sato and Rifkin 1988). In addition the release of bFGF was demonstrated using endotoxin in aortic endothelial cells and irradiation in bovine, porcine, and human endothelial cells (Gajdusek and Carbon 1989; Witte et al. 1989). Transient and reversible mechanically induced disruption of the plasma membrane caused the release of cytoplasmic bFGF from cultured endothelial cells (McNeil 1993; D'Amore 1990) and muscle fibres *in vivo* (Clarke et al. 1993). Although cell death could not be excluded in the above studies, in single, isolated, NIH 3T3 cells transfected with a cDNA encoding bFGF, bFGF is exocytosed into the extracellular medium and stimulates migration of the cells via an autocrine mechanism (Mignatti and Rifkin 1991; Mignatti et al. 1991). The release of aFGF from cultured cells by exocytosis has been observed to be promoted by heat shock (Jackson et al. 1992), suggesting a secretory pathway analogous to that proposed for interleukin-1 $\beta$  (Rubartelli et al. 1990).

As a result, two alternative pathways have been suggested for the release of bFGF by cells: transient cell wounding and exocytosis. Evidence for the transient cell wounding pathway (McNeil 1993) has been obtained from studies *in vivo* (Clarke et al. 1993) and in normal cultured cells (D'Amore 1990). However, the contribution of cell death to the observed release of bFGF cannot be ruled out. The evidence for the exocytotic pathway has been obtained from the investigation of the release of bFGF and aFGF from single cells under conditions where cell death is unlikely to be a contributing factor (Jackson et al. 1992; Mignatti et al. 1992; Mignatti and Rifkin 1991). One attraction of the exocytotic pathway is that it does not require mechanical stress to break the cell and hence it may operate in situations such as tissue development where the spatial and temporal regulation of the release of bFGF are important and yet there is no evidence for transient cell wounding.

## bFGF and its receptor in tumour diagnosis and its potential clinical applications

bFGF expression has been demonstrated in a number of neoplastic cell types, both in cultured cells and in tissues. bFGF expression by tumour cells is not only significant in view of a possible increase in tumour angiogenesis, but may also serve as an autocrine growth stimulation for the tumour cells themselves. bFGF has been detected in adrenal pheochromocytoma and chemodectoma (Statuto et al. 1993), renal cell carcinoma (Singh et al. 1994), bladder carcinoma (Allen and Maher 1993), brain tumours and astrocytomas (Li et al. 1994; Brem et al. 1992; Zagzag et al. 1990), hepatocellular carcinoma (Li et al. 1994), breast carcinoma (Anandappa et al. 1994; Singh et al. 1996; Bansal et al. 1995; Wilkinson et al. 1993), and carcinoma of the digestive tract (Ueki et al. 1995; Li et al. 1994; Ohtani et al. 1993). bFGF mRNA was detectable in metastatic primary invasive melanoma, but not in melanoma *in situ* and in benign melanocyte nevi (Reed et al. 1994). Cultured melanocytes do not express bFGF but are dependent on this factor for their growth. Cells derived from all other stages of melanoma progression (dysplastic nevus, primary melanoma metastasis) all express bFGF and show a decreasing dependency on exogenously added bFGF for their growth *in vitro* (Shih and Herlyn 1993; Rodeck et al. 1991). Cell lines derived from primary melanoma and melanoma metastasis do

not, in fact, require any exogenous bFGF at all for their growth, thereby illustrating the capacity of bFGF to serve as an autocrine growth factor (Rodeck et al. 1991). Recent work in our laboratory indicates a causal relationship between production of endogenous bFGF and invasion in human colon carcinoma (Galzie et al. 1997). We have examined two series of related human colon carcinoma cell lines that exhibit different invasive potentials when grown on collagen gels. The more invasive cells proliferate rapidly and produce more endogenous bFGF activity. In all cell lines, both growth and invasion are enhanced by exogenous bFGF and inhibited by anti-bFGF antibody.

A major activity of bFGF that could affect tumour growth is their angiogenic activity. Several studies have shown that FGFs make up a part of tumour angiogenesis factor, the cocktail of factors secreted by tumours that initiates neovascularization and nurtures the tumour (Folkman et al. 1988; Klagsbrun et al. 1976). Attempts at inhibiting this activity through the use of neutralizing antibodies to bFGF have had mixed results. Reilly et al. (1989) showed inhibition of angiogenesis caused by bFGF by using neutralizing antibodies in a kidney capsule model. In this model, the angiogenesis is caused strictly by bFGF added exogenously. Matsuzaki et al. (1989) used a more demanding model. They injected hybridomas secreting bFGF-neutralizing antibodies into athymic mice and found that these hybridomas grew into well-vascularized solid tumours. The implication of these experiments is that the activity of tumour angiogenesis factor, as a complex mixture of factors, is not affected by the removal of only one of the components; the remaining components can still cause neovascularization. Deletion of the target cells of tumour angiogenesis factor may overcome this problem and provide a more effective approach to inhibiting angiogenesis. Merwin et al. (1992) proposed the use of an aFGF – pseudomonas exotoxin fragment fusion protein to target neovascularizing endothelial cells and demonstrated that the fusion protein is cytotoxic against proliferating endothelial cells in tissue culture.

Recent studies have examined the role of FGFRs in tumour growth. A major focus of these studies has been breast carcinoma. Adnane et al. (1991) examined, by Southern blotting, the possible amplification of FGFR1 and FGFR2 genes in breast tumour tissues from over 350 patients. In approximately 10%, gene amplification was observed. Luqmani et al. (1992) used PCR analysis to examine normal and tumour breast cells for FGF and FGF receptor mRNA. They determined that FGFR1 and FGFR2 expression was variable among the samples (as was bFGF expression), but that variant forms of the receptors were being expressed that could influence the course of the disease.

Screening of breast tumour cell lines or tissues for FGFR4 message has shown overexpression of FGFR4 mRNA (Penault-Llorca et al. 1995; Ron et al. 1993), suggesting that FGFR4 may have a role in human mammary cancer. Coombes and co-workers suggested that membrane ruffling, which is a response to FGFR4 activation in some breast tumour cells, is associated with cell motility and could be important in determining metastatic potential in this tumour type (Johnston et al. 1995). Expression of FGFR4 may provide a mechanism for targeting breast tumour cells.

The high-affinity receptors are widely expressed in the embryo, but the expression is greatly reduced and restricted at

birth (Peters et al. 1993; Patstone et al. 1993). Even this restricted expression may be misleading, because the data are primarily from detection of message, which does not always correspond to active receptor on the cell surface, or even immunoreactivity (Yazaki et al. 1993). Receptor protein has been shown to be distributed in the cytoplasm, in the nucleus, and as a soluble form in serum and has even been reported to exist on the cell surface in an inactive form (Morrison et al. 1994). In addition, conclusions about receptor expression drawn from analysis of cells in culture may be misleading, because FGF receptor expression may be a requirement for stability in culture. As such, receptor expression in culture may poorly reflect the expression in vivo. Because good antibodies recognizing the individual forms of the receptors are only appearing now, the receptor isoform distribution in vivo is unclear. However, the early data indicate that tumour targeting through the FGF receptor can be achieved.

## Summary

Fibroblast growth factor tyrosine kinase receptors are encoded by four genes, but alternate splicing can result in more than 100 possible protein sequences. The ligand–receptor relationship is complex, owing to the diversity of the receptors and the large number of possible ligands; there are now nine (and probably more) members of the fibroblast growth factor family. This complicated ligand–receptor relationship creates many options to target cell types through the use of individual ligands or receptor-specific monoclonal antibodies. In vivo data demonstrate that FGF receptors are expressed on tumour cells and can be used to target tumours for growth inhibition. Given the complexity, it is possible that a unique targetable FGF receptor isoform can be found in one or more tumour types.

## Acknowledgments

The authors thank the North West Cancer Research Fund (Liverpool, U.K.) for supporting the work in their laboratory.

## References

- Acland, P., Dixon, M., Peters, G., and Dickson, C. 1990. Subcellular fate of the *int-2* oncoprotein is determined by choice of initiation codon. *Nature (London)*, **343**: 662–665.
- Adnane, J., Gaudray, P., Dionne, C.A., Crumley, G., Jay, M., Schlessinger, J., Jeanteur, P., Birnbaum, D., and Theillet, C. 1991. BEK and FLG, two receptors to members of the FGF family, are amplified in subsets of human breast cancers. *Oncogene*, **6**: 659–663.
- Ago, H., Kitagawa, Y., Fujishima, A., Matsura, Y., and Katsube, Y. 1991. Crystal structure of basic fibroblast growth factor at 1.6 Å resolution. *J. Biochem. (Tokyo)*, **110**: 360–363.
- Allen, L.E., and Maher, P.A. 1993. Expression of basic fibroblast growth factor and its receptor in an invasive bladder carcinoma cell line. *J. Cell. Physiol.* **155**: 368–375.
- Amalric, F., Bouche, G., Bonnet, H., Brethenou, P., Roman, A.M., Truchet, I., and Quarto, N. 1994. Fibroblast growth factor-2 (FGF-2) in the nucleus-translocation process and targets. *Biochem. Pharmacol.* **7**: 111–115.
- Anandappa, S.Y., Winstanley, J.H.R., Leinster, S., Green, B., Rudland, P.S., and Barraclough, R. 1994. Comparative expression of fibroblast growth factor messenger RNAs in benign and malignant breast disease. *Br. J. Cancer*, **69**: 772–776.
- Anderson, D., Koch, C.A., Grey, L., Ellis, C., Moran, M.F., and

- Pawson, T. 1990. Binding of SH2 domains of phospholipase-C-gamma-1, GAP and src to activated growth factor receptors. *Science* (Washington, D.C.), 250: 979-982.
- Andres, J.L., Defalcis, D., Noda, M., and Massague, J. 1992. Binding of two growth factor families to separate domains of the proteoglycan betaglycan. *J. Biol. Chem.* 267: 5927-5930.
- Baciu, P.C., Acaster, C., and Goetinck, P.F. 1994. Molecular-cloning and genomic organization of chicken syndecan-4. *J. Biol. Chem.* 269: 696-703.
- Baird, A., and Ling, N. 1987. Fibroblast growth factors are present in the extracellular matrix produced by endothelial cells in vitro: implication for a role of heparinase-like enzymes in the neovascular response. *Biochem. Biophys. Res. Commun.* 142: 428-435.
- Baird, A., Schubert, D., Ling, N., and Guillemain, R. 1988. Receptor- and heparin-binding domains of basic fibroblast growth factor. *Proc. Natl. Acad. Sci. U.S.A.* 85: 2324-2328.
- Baldin, V., Roman, A.M., Boscbierne, I., Amalric, F., and Bouche, G. 1990. Translocation of bFGF to the nucleus is G1 phase cell-cycle specific in bovine aortic endothelial-cells. *EMBO J.* 9: 1511-1517.
- Bansal, G.S., Yiangou, C., Coope, R.C., Gomm, J.J., Luqmani, Y.A., Coombes, R.C., and Johnston, C.L. 1995. Expression of fibroblast growth factor I is lower in breast cancer than in normal human breast. *Br. J. Cancer*, 72: 1420-1426.
- Barracough, R., Fernig, D.G., Rudland, P.S., and Smith, J.A. 1990. Synthesis of basic fibroblast growth factor upon differentiation of rat mammary epithelial to myoepithelial-like cells in culture. *J. Cell. Physiol.* 144: 333-344.
- Bashkin, P., Doctrow, S., Klagsbrun, M., Svahn, C.M., Folkman, J., and Vlodavsky, I. 1989. Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. *Biochemistry*, 28: 1737-1743.
- Beitz, J.G., Davol-Lewis, P., Clark, J.W., Kato, J., Medina, M., Frackelton, A.R., Lappi, D.A., Baird, A., and Calabresi, P. 1992. Antitumour activity of basic FGF-saporin mitotoxin in vitro and in vivo. *Cancer Res.* 52: 227-230.
- Bouche, G., Gas, N., Prats, H., Baldin, V., Tauber, J.P., Teissie, J., and Amalric, F. 1987. Basic fibroblast growth-factor enters the nucleus and stimulates the transcription of ribosomal genes in ABAE cells undergoing G<sub>0</sub>->G<sub>1</sub> transition. *Proc. Natl. Acad. Sci. U.S.A.* 84: 6770-6774.
- Brem, S., Tsanacalis, A.M., Gately, S., Gross, J.L., and Herblin, W.F. 1992. Immunolocalization of basic fibroblast growth factor to the microvasculature of human brain tumours. *Cancer*, 70: 2673-2680.
- Brown, T.A., Bouchard, T., St. John, T., Wayner, E., and Carter, W.G. 1991. Human keratinocytes express a new CD44 core protein (CD44e) as a heparan-sulphate intrinsic membrane proteoglycan with additional exons. *J. Cell Biol.* 113: 207-221.
- Bunnag, P., Waddell, K.S., Varban, M.L., and Chiu, I. M. 1991. Transformed phenotype conferred to NIH/3T3 cells by ectopic expression of heparin-binding growth factor-I acidic fibroblast growth factor. *In Vitro Cell. Dev. Biol.* 27: 89-96.
- Burgess, W.H., and Maciag, T. 1989. The heparin-binding (fibroblast) growth factor family of proteins. *Annu. Rev. Biochem.* 58: 575-606.
- Carey, D.J., Evans, D.M., Stahl, R.C., Asundi, V.K., Conner, K.J., Garbes, P., and Cizmeci-Smith, G. 1992. Molecular-cloning and characterization of N-syndecan, a novel transmembrane heparan-sulphate proteoglycan. *J. Cell Biol.* 117: 191-201.
- Champion-Arnaud, P., Ronsin, C., Gilbert, E., Gesnel, M.C., Houssaint, E., and Breathnach, R. 1991. Multiple messenger RNAs code for proteins related to the BEK fibroblast growth factor receptor. *Oncogene*, 6: 979-987.
- Chana, A.K., and Smith, J.A. 1991. The timing of late signals for initiation of the cell cycle in fibroblasts. *Growth Regul.* 1: 100-104.
- Cheifetz, S., and Massague, J. 1989. Transforming growth factor- $\beta$  (TGF- $\beta$ ) receptor proteoglycan-cell-surface expression and ligand-binding in the absence of glycosaminoglycan chains. *J. Biol. Chem.* 264: 12 025 - 12 028.
- Cheon, H.G., Larochelle, W.J., Bottaro, D.P., Burgess, W.H., and Aaronson, S.A. 1994. High affinity binding sites for related fibroblast growth factor ligands reside within different receptor immunoglobulin like domains. *Proc. Natl. Acad. Sci. U.S.A.* 91: 989-993.
- Clarke, M.S.F., Khakee, R., and McNeil, P.L. 1993. Loss of cytoplasmic basic fibroblast growth factor from physiologically wounded myofibers of normal and dystrophic muscle. *J. Cell Sci.* 106: 121-133.
- Colemanmacik, S., and Rosen, J.M. 1994. Differential temporal and spatial gene expression of fibroblast growth factor family members during mouse mammary gland development. *Mol. Endocrinol.* 8: 218-229.
- Cuevas, P., Burgos, J., and Baird, A. 1988. Basic fibroblast growth-factor (FGF) promotes cartilage repair in vivo. *Biochem. Biophys. Res. Commun.* 156: 611-618.
- D'Amore, P. 1990. Modes of FGF release in vivo and in vitro. *Cancer Metastasis Rev.* 9: 227-238.
- David, G., Lories, V., Decock, B., Marynen, P., Cassiman, J.J., and Van den Berghe, H. 1990. Molecular cloning of a phosphatidylinositol-anchored membrane heparan sulphate proteoglycan from human lung fibroblasts. *J. Cell Biol.* 111: 3165-3176.
- Davies, B.R., Fernig, D.G., Barracough, R., and Rudland, P.S. 1996. Effects on tumorigenicity and metastasis of transfection of a diploid benign rat mammary epithelial cell line with DNA corresponding to the mRNA for basic fibroblast growth factor. *Int. J. Cancer*, 65: 104-111.
- Dell, K.R., and Williams, L.T. 1992. A novel form of fibroblast growth factor receptor-2-alternative splicing of the 3rd immunoglobulin-like domain confers ligand binding specificity. *J. Biol. Chem.* 267: 21 225 - 21 229.
- Delli-Bovi, P.D., Curatola, A.M., Newman, K.M., Sato, Y., Moscatelli, D., Hewick, R.M., Rifkin, D.B., and Basilico, C. 1988. Processing, secretion and biological properties of a novel growth factor of the fibroblast growth factor family with oncogenic potential. *Mol. Cell. Biol.* 8: 2933-2941.
- Dickson, C., and Peters, G. 1987. Potential oncogene product related to growth factors. *Nature (London)*, 326: 833.
- Dickson, C., Smith, R., Brookes, S., and Peters, G. 1984. Tumorigenesis by mouse mammary tumour virus: proviral activation of a cellular gene in the common integration region int-2. *Cell*, 37: 529-536.
- Duan, D.S.R., Werner, S., and Williams, L.T. 1992. A naturally occurring secreted form of fibroblast growth factor (FGF) receptor-1 binds basic FGF in preference over acidic FGF. *J. Biol. Chem.* 267: 16 076 - 16 080.
- Eriksson, A.E., Cousens, L.A., Weaver, L.H., and Matthews, B.W. 1991. Three dimensional structure of human basic fibroblast growth factor. *Proc. Natl. Acad. Sci. U.S.A.* 88: 3441-3445.
- Esch, F., Baird, A., Ling, N., Ueno, N., Hill, F., Denory, L., Klepper, R., Gospodarowicz, D., Bolen, P., and Guillemain, R. 1985. Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino terminal sequence of bovine brain acidic FGF. *Proc. Natl. Acad. Sci. U.S.A.* 82: 6507-6511.
- Fantl, W.J., Johnson, D.E., and Williams, L.T. 1993. Signalling by receptor tyrosine kinases. *Annu. Rev. Biochem.* 62: 453-481.
- Fernig, D.G., and Gallagher, J.T. 1994. Fibroblast growth factors: an information network controlling tissue growth, morphogenesis and repair. *Prog. Growth Factor Res.* 5: 353-377.
- Fernig, D.G., Rudland, P.S., and Smith, J.A. 1992. Modulation of bFGF action by low affinity receptors in rat mammary myoepithelial-like cells in culture. *Growth Factors*, 5: 27-39.

- Fernig, D.G., Barraclough, R., Ke, Y., Wilkinson, M.C., Rudland, P.S., and Smith, J.A. 1993. Ectopic production of heparin-binding growth factors and receptors for basic fibroblast growth factor by rat mammary epithelial cell lines derived from malignant metastatic tumours. *Int. J. Cancer*, **54**: 629-635.
- Finch, P.W., Rubin, J.S., Miki, T., Ron, T., and Aaronson, S.A. 1989. Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science (Washington, D.C.)*, **241**: 752-755.
- Flaumenhaft, R., Moscatelli, D., Saksela, O., and Rifkin, D.B. 1989. Role of extracellular matrix in the action of basic fibroblast growth factor: matrix as a source of growth factor for long-term stimulation of plasminogen activator production and DNA synthesis. *J. Cell. Physiol.* **140**: 75-81.
- Florkiewicz, R.Z., and Sommer, A. 1989. Human basic fibroblast growth factor gene encodes four polypeptides: three initiate translation from non-AUG codons. *Proc. Natl. Acad. Sci. U.S.A.* **86**: 3978-3981.
- Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M.G., Ingber, D., and Vlodavsky, I. 1988. A heparin binding angiogenic protein basic fibroblast growth factor is stored within basement membrane. *Am. J. Pathol.* **130**: 393-400.
- Gajdusek, C.M., and Carbon, S. 1989. Injury-induced release of basic fibroblast growth factor from bovine aortic endothelium. *J. Cell. Physiol.* **139**: 570-579.
- Gallagher, J.T., Turnbull, J.E., and Lyon, M. 1990. Heparan sulphate proteoglycans. *Biochem. Soc. Trans.* **18**: 207-209.
- Galzie, Z., Fernig, D.G., Smith, J.A., Poston, G.J., and Kinsella, A. R. 1997. Invasion of human colorectal carcinoma cells is promoted by endogenous basic fibroblast growth factor. *Int. J. Cancer*, **71**: 390-395.
- Gomm, J.J., Smith, J., Ryall, G.K., Baillie, R., Turnbull, L., and Coombes, R.C. 1991. Localization of basic fibroblast growth factor and transforming growth factor- $\beta$ 1 in the human mammary gland. *Cancer Res.* **51**: 4685-4692.
- Gonzalez, A.M., Buscaglia, M., Ong, M., and Baird, A. 1990. Distribution of basic fibroblast growth factor in the 18-day rat fetus: localisation in the basement membranes of diverse tissues. *J. Cell Biol.* **110**: 753-765.
- Gospodarowicz, D. 1974. Localisation of fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. *Nature (London)*, **249**: 123-129.
- Gospodarowicz, D., and Chen, J. 1986. Heparin protects basic and acidic FGF from inactivation. *J. Cell. Physiol.* **128**: 475-485.
- Gospodarowicz, D., Bialecki, H., and Greenburg, G. 1978. Purification of fibroblast growth factor activity from bovine brain. *J. Biol. Chem.* **253**: 3736-3743.
- Gospodarowicz, D., Neufeld, G., and Schweigerer, L. 1986. Molecular and biological characterization of fibroblast growth-factor, an angiogenic factor which also controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells. *Cell Differ.* **19**: 1-17.
- Gospodarowicz, D., Ferrara, N., Schweigerer, L., and Neufeld, G. 1987. Structural characterization and biological functions of fibroblast growth factor. *Endocr. Rev.* **8**: 95-114.
- Gould, S.E., Upholt, W.B., and Kosher, R.A. 1992. Syndecan-3: a member of the syndecan family of membrane intercalated proteoglycans that is expressed in high amounts at the onset of chicken limb cartilage differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **89**: 3271-3275.
- Guimond, S., Maccarana, M., Olwin, B.B., Lindahl, U., and Rapraeger, A.C. 1993. Activating and inhibitory heparin sequences for FGF-2 (basic FGF): distinct requirements for FGF-1, and FGF-4. *J. Biol. Chem.* **268**: 23 906 - 23 914.
- Haigler, H.T., Maxfield, F.R., Willingham, M.C., and Pastan, I. 1980. Dansylcadaverine inhibits internalization of  $^{125}$ I-EGF in BALBc 3T3 cells. *J. Biol. Chem.* **255**: 1239-1241.
- Harper, J.W., and Lobb, R.R. 1988. Reductive methylation of lysine residues in acidic fibroblast growth factor: effect on mitogenic activity and heparin affinity. *Biochemistry*, **27**: 671-678.
- Hawker, J.R., and Granger, H.J. 1992. Internalized basic fibroblast growth factor translocates to nuclei of venular endothelial cells. *Am. J. Physiol.* **262**: 1525-1537.
- Heremans, A., Van Den Schueren, B., De Cock, B., Paulsson, M., Cassiman, J.J., Van Den Berghe, H., and David, G. 1989. Matrix associated heparan-sulfate proteoglycans core protein specific monoclonal antibodies decorate the pericellular matrix of connective tissue cells and the stromal side of basement membranes. *J. Cell Biol.* **109**: 3199-3211.
- Houssaint, E., Blanquet, P.R., Champion-Arnaud, P., Gesnel, M.C., Torriglia, A., Courtois, Y., and Breathnach, R. 1990. Related growth factors receptor genes exist in the human genome. *Proc. Natl. Acad. Sci. U.S.A.* **87**: 8180-8184.
- Imamura, T., Tokita, Y., and Mitsui, Y. 1992. Identification of a heparin-binding growth factor-1 nuclear translocation sequence by deletion mutation analysis. *J. Biol. Chem.* **267**: 5676-5679.
- Isacchi, A., Statuto, M., Chiesa, R., Bergonzoni, L., Rusnati, M., Sarmientos, P., Ragnotti, G., and Presta, M. 1991. A six-amino acid deletion in basic fibroblast growth factor dissociates its mitogenic activity from its plasminogen activator inducing capacity. *Proc. Natl. Acad. Sci. U.S.A.* **88**: 2628-2632.
- Jackson, A., Friedman, S., Zhan, X., Engleka, K.A., Forough, R., and Maciag, T. 1992. Heat shock induces the release of fibroblast growth factor-1 from NIH-3T3 cells. *Proc. Natl. Acad. Sci. U.S.A.* **89**: 10 691 - 10 695.
- Jalkanen, M., Rapraeger, A., and Bernfield, M. 1988. Mouse mammary epithelial cells produce basement membrane and cell surface heparan sulphate proteoglycans containing distinct core proteins. *J. Cell Biol.* **106**: 953-962.
- Jaye, M., Schlessinger, J., and Dionne, C.A. 1992. Fibroblast growth factor receptor tyrosine kinases-molecular analysis and signal transduction. *Biochim. Biophys. Acta*, **1135**: 185-199.
- Johnston, C.L., Co, H.C., Gom, J.J., and Coombes, R.C. 1995. bFGF and aFGF induce membrane ruffling in breast cancer cells but not in normal breast epithelial cells: FGFR-4 involvement. *Biochem. J.* **306**: 609-616.
- Jouanneau, J., Moens, G., Bourgeois, Y., Poupon, M.F., and Thiery, J.P. 1994. A minority of carcinoma cells producing acidic fibroblast growth factor induces a community effect for tumour progression. *Proc. Natl. Acad. Sci. U.S.A.* **91**: 286-290.
- Kallunki, P., and Tryggvason, K. 1992. Human basement membrane heparan sulphate proteoglycan core protein: a 467kD protein containing multiple domains resembling elements of the low density lipoprotein receptor, laminin, neural cell adhesion molecules, and epidermal growth factor. *J. Cell Biol.* **116**: 559-571.
- Kan, M., DiSorbo, D., Hou, J., Mansson, P.E., and McKeehan, W.L. 1988. High and low affinity binding of heparin-binding growth factor to a 130 kDa receptor correlates with stimulation and inhibition of growth of a differentiated human hepatoma cell. *J. Biol. Chem.* **263**: 11 306 - 11 313.
- Keegan, K., Meyer, S., and Hayman, M.J. 1991. Structural and biosynthetic characterization of the fibroblast growth factor receptor-3 (FGFR-3) protein. *Oncogene*, **6**: 2229-2236.
- Kiefer, M.C., Stephan, J.C., Crawford, K., Okino, K., and Barr, P.J. 1990. Ligand-affinity cloning and structure of a cell surface heparan sulphate proteoglycan that binds basic fibroblast growth factor. *Proc. Natl. Acad. Sci. U.S.A.* **87**: 6985-6989.
- Kimmelman, D., Abraham, J., Haaparanta, T., Palisi, T., and Kirschner, M. 1988. The presence of fibroblast growth factor in the frog egg: its role as a natural mesoderm inducer. *Science (Washington, D.C.)*, **242**: 1053-1056.
- Klagsbrun, M. 1989. The fibroblast growth factor family: structural and biological properties. *Prog. Growth Factor Res.* **1**: 207-235.

- Klagsbrun, M., Knighton, D., and Folkman, J. 1976. Tumour angiogenesis activity in cells grown in tissue culture. *Cancer Res.* 36: 110-114.
- Klotz, I.M. 1982. Numbers of receptor sites from Scatchard graphs: facts and fantasies. *Science (Washington, D.C.)*, 217: 1247-1249.
- Koda, J.E., Rapraeger, A., and Bernfield, M. 1985. Heparan sulphate proteoglycans from mouse mammary epithelial cells: surface proteoglycan as a receptor for interstitial collagens. *J. Biol. Chem.* 260: 8157-8162.
- Kojima, T., Leone, C.W., Marchildon, G.A., Marcum, J.A., and Rosenberg, R.D. 1992. Isolation and characterization of heparan sulfate proteoglycans produced by cloned rat microvascular endothelial cells. *J. Biol. Chem.* 267: 4859-4869.
- Lane, D., Flynn, A.M., Gallagher, J., Scully, M.F., and Lindahl, U. 1989. Size dependant anticoagulant activities of glycosaminoglycans with low affinity for antithrombin (dermatan sulphate and low affinity heparin). *Br. J. Haematol.* 73: 440.
- Li, D., Bell, J., Brown, A., and Berry, C.L. 1994. The observation of angiogenin and basic fibroblast growth factor gene expression in human colonic adenocarcinomas, gastric adenocarcinomas, and hepatocellular carcinomas. *J. Pathol.* 172: 171-175.
- Lobb, R.R. 1988. Thrombin inactivates acidic fibroblast growth factor but not basic fibroblast growth factor. *Biochemistry*, 27: 2572-2578.
- Luqmani, Y.A., Graham, M., and Coombes, R.C. 1992. Expression of basic fibroblast growth factor, FGFR1 and FRFR2 in normal and malignant human breast, and comparison with other human tissues. *Br. J. Cancer*, 66: 273-280.
- Mali, M., Jaakkola, P., Arvilommi, A.M., and Jalkanen, M. 1990. Sequence of human syndecan indicates a novel gene family of integral membrane proteoglycans. *J. Biol. Chem.* 265: 6884-6889.
- Mansukhani, A., Deller, P., Moscatelli, D., Kornbluth, S., Hanafusa, H., and Basilico, C. 1992. Characterization of the murine BEK fibroblast growth factor (FGF) receptor-activation by 3 members of the FGF family and requirement of heparin. *Proc. Natl. Acad. Sci. U.S.A.* 89: 3305-3309.
- Marics, I., Adelaide, J., Raybaud, F., Mattei, M.G., Coulier, F., Planche, J., de Lapeyriere, O., and Birnbaum, D. 1989. Characterization of HST-related FGF 6 gene, a new member of the fibroblast growth factor family. *Oncogene*, 4: 335-340.
- Marynen, P., Cassiman, J., Van den Berghe, H., and David, G. 1989. Partial primary structure of the 48 kilodalton and 90 kilodalton core protein of cell surface associated heparan sulphate proteoglycans of lung fibroblast: prediction of an integral membrane domain and evidence for multiple distinct core proteins at the cell surface of human lung fibroblasts. *J. Biol. Chem.* 264: 7017-7024.
- Matsuzaki, K., Yoshitake, Y., Matuo, Y., Sasaki, H., and Nishikawa, K. 1989. Monoclonal antibodies against heparin binding growth factor ii/basic fibroblast factor that block its biological activity: Invalidity of the antibodies for tumour angiogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 86: 9911-9915.
- McKeehan, W.L., and Crabb, J.W. 1987. Isolation and characterization of different molecular and characterization of heparin binding growth factor-I from bovine brain. *Anal. Biochem.* 164: 563-569.
- McNeil, P.L. 1993. Cellular and molecular adaptations to injurious mechanical stress. *Trends Cell Biol.* 3: 302-307.
- Merwin, J.R., Lynch, M.J., Madri, J.A., Pastan, I., and Siegall, C.B. 1992. Acidic fibroblast growth factor: Pseudomonas exotoxin chimeric protein elicits antiangiogenic effects on endothelial cells. *Cancer Res.* 52: 4995-5001.
- Mignatti, P., and Rifkin, D.B. 1991. Release of basic fibroblast growth factor, an angiogenic factor devoid of secretory signal sequence: a trivial phenomenon or a novel secretion mechanism. *J. Cell. Biochem.* 4: 201-207.
- Mignatti, P., Morimoto, T., and Rifkin, D.B. 1991. Basic fibroblast growth factor, released by single, isolated cells stimulates their migration in an autocrine manner. *Proc. Natl. Acad. Sci. U.S.A.* 88: 11 007 - 11 011.
- Mignatti, P., Morimoto, T., and Rifkin, D.B. 1992. Basic fibroblast growth factor, a protein devoid of secretory signal sequence, is released by cells via a pathway independent of the endoplasmic reticulum-golgi complex. *J. Cell. Physiol.* 151: 81-93.
- Miyamoto, M., Naruo, K., Seko, C., Matsumoto, S., Kondo, T., and Kurokawa, T. 1993. Molecular cloning of a novel cytokine cDNA encoding the 9th member of the fibroblast growth factor family, which has a unique secretion property. *Mol. Cell. Biol.* 13: 4251-4259.
- Morita, H., Shinzato, T., David, G., Mizutani, A., Habuchi, H., Fujita, Y., Ito, M., Asai, J., Maeda, K., and Kimata, K. 1994. Basic fibroblast growth factor binding domain of heparan sulphate in the human glomerulosclerosis and renal tubulointerstitial fibrosis. *Lab. Invest.* 71: 528-535.
- Morrison, R.S., Yamaguchi, F., Bruner, J.M., Tang, M., McKeehan, W.L., and Berger, M.S. 1994. Fibroblast growth factor receptor gene expression and immunoreactivity are elevated in human glioblastoma multiforme. *Cancer Res.* 54: 2794-2799.
- Moscatelli, D. 1987. High and low affinity binding sites for basic fibroblast growth factor on cultured cells: absence of a role for low affinity binding in the stimulation of plasminogen activator production by bovine capillary endothelial cells. *J. Cell. Physiol.* 131: 123-130.
- Moscatelli, D., Silverstein, J., Manojas, R., and Rifkin, D.B. 1987. Mr 25000 heparin binding protein from guinea pig brain is a high molecular weight form of basic fibroblast growth factor. *Proc. Natl. Acad. Sci. U.S.A.* 84: 5778-5782.
- Murdoch, A.D., Dodge, G.R., Cohen, I., Tuan, R.S., and Iozzo, R.V. 1992. Primary structure of the human heparan sulphate proteoglycan from basement membrane (HSPG2/perlecan): a chimeric molecule with multiple domain homologous to the low density lipoprotein receptor, laminin, neural cell adhesion molecules, and epidermal growth factor. *J. Biol. Chem.* 267: 8544-8557.
- Murgue, B., Tsunekawa, S., Rosenberg, I., Debeaumont, M., and Podolsky, D.K. 1994. Identification of a novel variant form of fibroblast growth factor receptor 3 (FGFR3 IIIb) in human colonic epithelium. *Cancer Res.* 54: 5206-5211.
- Musacchio, A., Gibson, T., Lehto, V.P., and Saraste, M. 1992. SH3: an abundant protein domain in search of a function. *FEBS Lett.* 307: 55-61.
- Myoken, Y., Myoken, Y., Okamoto, J., Kan, M., McKeehan, W.L., Sato, J.D., and Takada, K. 1996. Expression of fibroblast growth factor-1 (FGF-1), FGF-2 and FGF receptor-1 in a human salivary gland adenocarcinoma cell line: evidence of growth. *Int. J. Cancer*, 65: 650-657.
- Neufeld, G., and Gospodarowicz, D. 1985. The identification and partial characterization of the fibroblast growth factor receptor of baby hamster-kidney cells. *J. Biol. Chem.* 260: 13 860 - 13 868.
- Nonomura, N., Lu, J., Tanaka, A., Yamanishi, H., Sato, B., Sonoda, T., and Matsumoto, K. 1990. Interaction of androgen induced autocrine heparin binding growth factor with fibroblast growth factor receptor on androgen dependent Shionogi carcinoma 115 cells. *Cancer Res.* 50: 2316-2321.
- Noonan, D.M., Horigan, E.A., Ledbetter, S.R., Vogeli, G., Sasaki, M., Yamada, Y., and Hassell, J.R. 1988. Identification of cDNA clones encoding different domains of the basement membrane heparan sulphate proteoglycan. *J. Biol. Chem.* 263: 16 379 - 16 387.
- Ohtani, H., Nakamura, S., Wantanabe, Y., Mizoi, T., Saku, T., and Nagura, H. 1993. Immunocytochemical localization of basic fibroblast growth factor in carcinomas and inflammatory lesions of the human digestive tract. *Lab. Invest.* 68: 520-527.



- Olwin, B.B., and Hauschka, S.D. 1986. Identification of the fibroblast growth factor receptor of Swiss 3T3 cells and mouse skeletal muscle myoblasts. *Biochemistry*, **25**: 3487-3492.
- Ornitz, D.M., Yayon, A., Flanagan, J.G., Svah, C.M., Levi, E., and Leder, P. 1992. Heparin is required for cell free binding of basic fibroblast growth factor to a soluble receptor and for mitogenesis in whole cells. *Mol. Cell. Biol.* **12**: 240-247.
- Partanen, J., Makela, T.P., Eerola, E., Korhonen, J., Hirvonen, H., Claessonwelsh, L., and Alitalo, K. 1991. FGFR-4, a novel acidic fibroblast growth factor receptor with a distinct expression pattern. *EMBO J.* **10**: 1347-1354.
- Pasquale, E.B., and Singer, S.J. 1989. Identification of a developmentally regulated protein tyrosine kinase by using anti-phosphotyrosine antibodies to screen a cDNA expression library. *Proc. Natl. Acad. Sci. U.S.A.* **86**: 5449-5453.
- Patstone, G., Pasquale, E.B., and Maher, P.A. 1993. Different members of the fibroblast growth receptor family are specific to distinct cell types in the developing chicken embryo. *Dev. Biol.* **155**: 107-123.
- Penault-Llorca, F., Bertucci, F., Adelaide, J., Parc, P., Coulier, F., Lacquemier, J., Birnbaum, D., and DeLapeyriere, O. 1995. Expression of FGF and FGF receptor genes in human breast cancer. *Int. J. Cancer*, **61**: 170-176.
- Peters, K., Ornitz, D., Werner, S., and Williams, L.T. 1993. Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis. *Dev. Biol.* **155**: 423-430.
- Pierce, A., Lyon, M., Hampson, I.N., Cowling, G.J., and Gallagher, J.T. 1992. Molecular cloning of the major cell surface heparan sulphate proteoglycan from rat liver. *J. Biol. Chem.* **267**: 3894-3990.
- Pledger, W.J., Stiles, C.D., Antoniades, H.N., and Scher, C.D. 1978. An ordered sequence of events is required before BALB/C-T3 cells become committed to DNA synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **75**: 2839-2843.
- Powell, P.P., and Klagsbrun, M. 1991. Three forms of rat basic fibroblast growth factor are made from a single messenger RNA and localize to the nucleus. *J. Cell. Physiol.* **148**: 202-210.
- Prats, H., Kaghad, M., Prats, A.C., Klagsbrun, M., Lelias, J.M., Liauzun, P., Chalon, P., Tauber, J.P., Amalric, F., Smith, J.A., and Caput, D. 1989. High molecular mass forms of basic fibroblast growth factor are initiated by alternative CUG codons. *Proc. Natl. Acad. Sci. U.S.A.* **83**: 836-840.
- Presta, M., Rusnati, M., Urbinati, C., Sommer, A., and Ragnotti, G. 1991. Biologically active synthetic fragments of human basic fibroblast growth factor (bFGF): identification of two Asp-Gly-Arg-containing domains involved in the mitogenic activity of bFGF in endothelial cells. *J. Cell. Physiol.* **149**: 512-524.
- Presta, M., Statuto, M., Isacchi, A., Caccia, P., Pozzi, A., Gulandris, A., Rusnati, M., Bergonzoni, I., and Sarmientos, P. 1992. Structure function relationship of basic fibroblast growth factor site directed mutagenesis of a putative heparin-binding and receptor binding region. *Biochem. Biophys. Res. Commun.* **185**: 1098-1107.
- Prestrelski, S.J., Fox, G.M., and Arakawa, T. 1992. Binding of heparin to basic fibroblast growth induces a conformational change. *Arch. Biochem. Biophys.* **293**: 314-319.
- Rapraeger, A., Jalkanen, M., Endo, E., Koda, J., and Bernfield, M. 1985. The cell surface proteoglycan from mouse mammary epithelial cells bears chondroitin sulphate and heparan sulphate glycosaminoglycans. *J. Biol. Chem.* **260**: 11 046 - 11 052.
- Rapraeger, A.C., Krufka, A., and Olwin, B.B. 1991. Requirement of heparan sulphate for bFGF mediated fibroblast growth and myoblast differentiation. *Science (Washington, D.C.)*, **252**: 1705-1708.
- Reed, J.A., McNutt, N.S., and Albino, A.P. 1994. Differential expression of basic fibroblast growth factor (bFGF) in melanocytic lesions demonstrated by in situ hybridization. *Am. J. Pathol.* **144**: 329-336.
- Reiland, J., and Rapraeger, A.C. 1993. Heparan sulphate proteoglycan and FGF receptor target basic FGF to different intracellular destinations. *J. Cell Sci.* **105**: 1085-1093.
- Reilly, T.M., Taylor, D.S., Herblin, W.F., Thooles, M.J., Chiu, A.T., Watson, D.W., and Timmermans, P.B.M.W.M. 1989. Monoclonal antibodies directed against basic fibroblast growth factor which inhibits its biological activity in vitro and in vivo. *Biochem. Biophys. Res. Commun.* **164**: 736-743.
- Rifkin, D.B., and Moscatelli, D. 1989. Recent developments in the cell biology of basic fibroblast growth factor. *J. Cell Biol.* **109**: 1-6.
- Rodeck, U., Becker, D., and Herlyn, M. 1991. Basic fibroblast growth factor in human melanoma. *Cancer Cells*, **3**: 308-311.
- Ron, D., Reich, R., Chedid, M., Lengel, C., Cohe, O.E., Chan, A.M.L., Neufeld, G., Miki, T., and Tronick, S.R. 1993. Fibroblast growth factor receptor-4 is a high affinity receptor for both acidic and basic fibroblast growth factor but not for keratinocyte growth factor. *J. Biol. Chem.* **268**: 5388-5394.
- Rubartelli, A., Cozzolino, F., Talio, M., and Sitia, R. 1990. A novel secretory pathway for interleukin-1-beta, a protein lacking a signal sequence. *EMBO J.* **9**: 1503-1510.
- Rubin, J.S., Osada, H., Finch, P.W., Taylor, W.G., Rudikoff, S., and Aaronson, S.A. 1989. Purification and characterization of a newly identified growth factor specific for epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **86**: 802-806.
- Rudland, P.S., and Jimenez de Asua, L. 1979. Action of growth factors in the cell cycle. *Biochim. Biophys. Acta*, **560**: 91-133.
- Rudland, P.S., Seifert, W.E., and Gospodarowicz, D. 1974. Growth control and mitogenic response in cultured fibroblasts: induction of the pleiotypic and mitogenic by a purified growth factor. *Proc. Natl. Acad. Sci. U.S.A.* **71**: 2600-2604.
- Rudland, P.S., Platt-Higgins, A.M., Wilkinson, M.C., and Fernig, D.G. 1993. Immunocytochemical identification of basic fibroblast growth factor in the developing rat mammary gland: variations in location are dependent on glandular structure and differentiation. *J. Histochem. Cytochem.* **41**: 887-898.
- Sakamoto, H., Mori, M., Taira, M., Yoshida, T., Matsukawa, S., Shimizu, K., Sekiguchi, M., Terada, M., and Sugimura, T. 1986. Transforming gene from human stomach cancers and a non-cancerous portion of stomach mucosa. *Proc. Natl. Acad. Sci. U.S.A.* **83**: 3997-4001.
- Sato, Y., and Rifkin, D.B. 1988. Autocrine regulation of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. *J. Cell Biol.* **107**: 1199-1205.
- Saunders, S., and Bernfield, M. 1988. Cell surface proteoglycan binds mouse mammary epithelial cells to fibronectin and behaves as a receptor for interstitial matrix. *J. Cell Biol.* **106**: 423-430.
- Saunders, S., Jalkanen, M., O'Farrell, S., and Bernfield, M. 1989. Molecular cloning of syndecan, an integral membrane proteoglycan. *J. Cell Biol.* **108**: 1547-1556.
- Seno, M., Sasada, R., Kurokawa, T., and Igarashi, K. 1990. Carboxyl-terminal structure of basic fibroblast growth factor significantly contributes to its affinity for heparin. *Eur. J. Biochem.* **188**: 239-245.
- Shi, E., Kan, M., Xu, J., and McKeehan, W. 1991. A 16 kilodalton heparin binding (fibroblast) growth factor type one appears in a stable 40 kilodalton complex after receptor dependent internalization. *J. Biol. Chem.* **266**: 5774-5779.
- Shih, I.M., and Herlyn, M. 1993. Role of growth factors and their receptors in the development and progression of melanoma. *J. Invest. Dermatol.* **100**: 196S-203S.
- Singh, R.K., Bucana C.D., Gutman, M., Fan, D., Wilson, M.R., and Fidler, I.J. 1994. Organ site-dependent expression of basic fibroblast growth factor in human renal cell carcinoma cells. *Am. J. Pathol.* **145**: 365-374.
- Singh, G.P., Green, B., Galzie, Z., Poston, G.J., Sutton, R., Taylor, B.A., and Kinsella, A.R. 1996. Expression of basic



- fibroblast growth factor in colorectal carcinoma. *Int. J. Oncol.* 8: 191-195.
- Slack, J.M.W., Isaacs, H.V., and Darlington, B.G. 1988. Inductive effects of FGF and lithium ion on *Xenopus* blastula ectoderm. *Development (Cambridge)*, 103: 581-590.
- Smith, R., Peters, G., and Dickson, C. 1988. Expressed from the int-2 gene in mouse embryonal carcinoma cell lines encode a protein with homology to fibroblast growth factors. *EMBO J.* 7: 1013-1022.
- Songyang, Z., Shoelson, S.E., McGlade, J., Oliver, P., Pawson, T., Bustelo, X.R., Barbacid, M., Sabe, H., Hanafusa, H., Yi, T., Ren, R., Baltimore, D., Ratnoffsky, S., Feldman, R.A., and Cantley, L.C. 1994. Specific motifs recognized by the SH2 domains of CSK, 3BP2, FPS, FES, GRB-2, HCP, SHC, SYK and VAV. *Mol. Cell. Biol.* 14: 2777-2785.
- Stauto, M., Ennas, M.G., Zamboni, G., Bonetti, F., Pea, M., Bernardello, F., Pozzi, A., Rusnati, M., Gualandris, A., and Presta, M. 1993. Basic fibroblast growth factor in human pheochromocytoma: a biochemical and immunohistochemical study. *Int. J. Cancer*, 53: 5-10.
- Taira, M., Yoshida, T., Sakamoto, H., Miyagawa, K., Sakamoto, H., Terada, M., and Sugimura, T. 1987. cDNA sequences of human transforming gene hst and identification of the coding sequence required for transforming activities. *Proc. Natl. Acad. Sci. U.S.A.* 84: 2980-2984.
- Tanaka, A., Miyamoto, K., Minamino, N., Takeda, M., Sato, B., Matsuo, H., and Matsumoto, K. 1992. Cloning and characterization of an androgen induced growth factor essential for the androgen dependent growth of mouse mammary carcinoma cells. *Proc. Natl. Acad. Sci. U.S.A.* 89: 8928-8932.
- Taylor, W.R., Greenberg, A.H., Turley, E.A., and Wright, J.A. 1993. Cell motility, invasion and malignancy induced by overexpression of K-FGF or bFGF. *Exp. Cell Res.* 204: 295-301.
- Tessler, S., and Neufeld, G. 1990. Basic fibroblast growth factor accumulates in the nuclei of various bFGF producing cell types. *J. Cell. Physiol.* 145: 310-317.
- Thomas, K.A., Riley, M.C., Lemmon, S.K., Baglan, N.C., and Bradshaw, R. 1980. Brain fibroblast growth factor. *J. Biol. Chem.* 255: 5517-5520.
- Turnbull, J.E., and Gallagher, J.T. 1988. Oligosaccharide mapping of heparan sulphate by polyacrylamide gradient gel electrophoresis and electrotransfer to nylon membrane. *Biochem. J.* 251: 597-608.
- Turnbull, J.E., Fernig, D.G., Ke, Y.Q., Wilkinson, M.C., and Gallagher, J.T. 1992. Identification of the basic fibroblast growth factor binding sequence in fibroblast heparan sulphate. *J. Biol. Chem.* 267: 10 337 - 10 341.
- Ueki, T., Koji, T., Tamiya, S., Nakane, P.K., and Tsuneyoshi, M. 1995. Expression of basic fibroblast growth factor and fibroblast growth factor receptor in advanced gastric carcinoma. *J. Pathol.* 177: 353-361.
- Ullrich, A., and Schlessinger, J. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell*, 61: 203-212.
- Vainikka, S., Partanen, J., Bellosta, P., Coulier, F., Basilico, C., Jaye, M., and Alitalo, K. 1992. Fibroblast growth factor receptor-4 shows novel features in genomic structure, ligand binding and signal transduction. *EMBO J.* 11: 4273-4280.
- Vlodavsky, I., Folkman, J., Sullivan, R., Fridman, R., Ishai-Michaeli, R., Sasse, J., and Klagsbrun, M. 1987. Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition in to subendothelial extracellular matrix. *Proc. Natl. Acad. Sci. U.S.A.* 84: 2292-2296.
- Vlodavsky, I., Bar-Shavit, R., Ishai-Michaeli, R., Bhaskin, P., and Fuks, Z. 1991. Extracellular sequestration and release of fibroblast growth factor. A regulatory mechanism? *Trends Biochem. Sci.* 16: 268-271.
- Wallicke, P.A., Feige, J.J., and Baird, A. 1989. Characterization of the neuronal receptor for basic fibroblast growth factor and comparison to receptors on mesenchymal cells. *J. Biol. Chem.* 264: 4120-4126.
- Walker, A., Turnbull, J.E., and Gallagher, J.T. 1994. Specific heparan sulphate saccharides mediate the activity of basic fibroblast growth factor. *J. Biol. Chem.* 269: 931-935.
- Werner, S., Duan, D.S.R., Duvries, C., Peters, K.G., Johnson, D.E., and Williams, L.T. 1992. Differential splicing in the extracellular region of fibroblast growth factor receptor-1 generates receptor variants with different ligand binding specificities. *Mol. Cell. Biol.* 12: 82-88.
- Whalen, G.F., Shing, Y., and Folkman, J. 1989. The fate of intravenously administered bFGF and the effect of heparin. *Growth Factors*, 1: 157-164.
- Wiedlocha, A., Falnes, P.O., Madhus, I.H., Sandvig, K., and Olsnes, S. 1994. Dual mode of signal transduction by externally added acidic fibroblast growth factor. *Cell*, 76: 1039-1051.
- Wilkinson, M.C., Gardner, M.J., Gould, P.W., Taylor, B.A., and Fernig, D.G. 1993. Normal and malignant human colonic mucosa contain acidic and basic fibroblast growth factors. *Int. J. Oncol.* 3: 933-936.
- Witte, L., Fuks, Z., Haimovitz, F.A., Vlodavsky, I., Goodman, D.S., and Eldor, A. 1989. Effects of irradiation on the release of growth factors from cultured bovine, porcine, and human endothelial cells. *Cancer Res.* 49: 5066-5072.
- Yamaguchi, F., Saya, H., Bruner, J.M., and Morrison, R.S. 1994. Differential expression of two fibroblast growth factor receptor genes is associated with malignant progression in human astrocytomas. *Proc. Natl. Acad. Sci. U.S.A.* 91: 484-488.
- Yamaguchi, J.P., and Rossant, J. 1995. Fibroblast growth factor in mammalian development. *Curr. Opin. Genet. Dev.* 485-491.
- Yan, G.C., Wang, F., Fukabori, Y., Sussman, D., Hou, J.Z., and McKeehan, W.L. 1992. Expression and transforming activity of a variant of the heparin binding fibroblast growth factor receptor (flg) gene resulting from splicing of the alpha exon at an alternate 3'-acceptor site. *Biochem. Biophys. Res. Commun.* 183: 423-430.
- Yanagishita, M., and Hascall, V.C. 1984. Metabolism of proteoglycans in rat ovarian granulosa cell culture: multiple intracellular degradative pathways and the effect of chloroquine. *J. Biol. Chem.* 259: 10 270 - 10 283.
- Yayon, A., Klagsbrun, M., Esko, J.D., Leder, P., and Ornitz, D.M. 1991. Cell surface, heparin like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell*, 64: 841-848.
- Yazaki, N., Fujita, H., Ohta, M., Kawasaki, T., and Itoh, N. 1993. The structure and expression of the FGF receptor-1 mRNA isoforms in rat tissues. *Biochim. Biophys. Acta*, 1172: 37-42.
- Yoshida, T., Miyagawa, K., Odagiri, H., Sakamoto, H., Little, P.F.R., Terada, M., and Sugimura, T. 1987. Genomic sequence of hst a transforming gene encoding a protein homologous to fibroblast growth factor and the int-2 encoded protein. *Proc. Natl. Acad. Sci. U.S.A.* 84: 7305-7309.
- Zagzag, D., Miller, D.C., Sato, Y., Rifkin, D.B., and Burstein, D.E. 1990. Immunohistochemical localization of basic fibroblast growth factor in astrocytomas. *Cancer Res.* 50: 7393-7398.
- Zhan, X., Bates, B., Hu, X., and Goldfarb, M. 1988. The human FGF-5 gene encodes a novel protein related to fibroblast growth factors. *Mol. Cell. Biol.* 8: 3487-3495.
- Zhang, J., Cousens, L.S., Barr, P.S., and Sprang, S.R. 1991. Three dimensional structure of human basic fibroblast growth factor, a structural homolog of interleukin-1 $\beta$ . *Proc. Natl. Acad. Sci. U.S.A.* 88: 3446-3450.
- Zimmer, Y., Givol, D., and Yayon, A. 1993. Multiple structural elements determine ligand binding of fibroblast growth factor receptors: evidence that both Ig domain 2 and domain 3 define receptor specificity. *J. Biol. Chem.* 268: 7899-7903.